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Ecology and behaviour of the Fallow deer (*Dama dama L. 1758*): an advanced structural equation models approach

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“Causa latet, vis est notissima."
Ovidio, *Metamorfosi*, IV, 287

SUMMARY

Here I present results of a long-term study (1991-2003) performed in the lekking fallow deer (*Dama dama*) population of Castelporziano Preserve, Rome, Italy, where data were collected through radio-tracking and direct observations. In addition I present the analysis of a 100-years-long time series (1886-2003) relative to the abundance of three sympatric species of wild ungulates (fallow deer, roe deer and wild boar).

New results on the behavioural ecology of the fallow deer, with special regard to mating strategies and success were reported and on statistical methodology.

First, I showed that in accordance to the "female choice" and "male dominance" in fallow deer the literature reports contrasting results. This variability may reflect actual differences among studied populations, but it may also be generated by methodological differences and statistical shortcomings in data analysis. I have carried out a review of the statistical methods used so far in lek studies that shows a prevalence of Linear Models (LM) and Generalized Linear Models (GLM) which may be affected by problems in inferring cause-effect relationships; multi-collinearity among explanatory variables and erroneous handling of non-normal and non-continuous distributions of the response variable. I have used a dataset on lekking fallow deer , to contrast the methods and procedures employed so far, and I propose a novel approach based on Generalized Structural Equations Models (GSEMs). GSEMs combine the power and flexibility of both SEM and GLM in a unified modeling framework. We showed that LMs fail to identify several important predictors of male copulatory success and yields very imprecise parameter estimates. Minor variations in data transformation yield wide changes in results and the method appears unreliable. GLMs improved the analysis, but GSEMs provided better results, because the use of latent variables decreases the impact of measurement errors. Using GSEMs, we were able to test contrasting hypotheses and calculate both direct and indirect effects, and we reached a high precision of the estimates, which implies a high predictive ability.

Second, I showed that female fallow deer which are less experienced (young) and/or incurring in higher travel costs (with a home range far from the lek), adopt indirect forms of mate selection more often than adult females or females resident near the lek. In particular, younger females remained longer in the lek and in the vicinity of bucks than adult ones, and returned to the lek after copulation. However, despite the time spent at the lek, young females were not able to select highest-rank bucks, and relied on *territory*

choice more often than adult does. Farther females visited the lek less frequently and arrived later than near females, but they were seen more often inside female groups. Surprisingly, we did not find a different amount of *copying* in young or in farther females. Our results can contribute to clarifying the co-evolution of mating strategies of both sexes in ungulate leks.

Third, I showed that in Mediterranean ungulate communities, exposed to relatively mild climate fluctuations, trophic interactions such as density dependence, competition and facilitation have stronger effects than environmental controls such as climate. The results of all analyses confirmed that intra-specific competition was a main ingredient in the regulation of the growth rates within the ungulate community at Castelporziano, in keeping with the view that density-independent regulation is more important in those populations facing strong climatic fluctuations.

The importance of this study lies in the fact that, to our knowledge, it is the first comparative study of SEM and GSEM models. In particular, the possibility of using SEMs to test hypotheses in competition and investigate both remote and proximate effects is of particular interest in ecological and evolutionary studies. A second original finding of this thesis is that our knowledge is the first study that deals with copulatory success by adult and subadult lekking does.

Finally I have proposed a novel approach based (SEMs) and (GSEMs) for testing the effects of climatic and environmental factors, of the density of potential competitors and density dependence on the growth rate of wild boar, fallow and roe deer for the whole study period, in order to test the hypothesis that density dependence and competition are more relevant than climatic and environmental factors in regulating Mediterranean ungulate populations, in contrast with ungulates living in harsher and more fluctuating environments.

RIASSUNTO

In questa tesi presento i risultati di uno studio a lungo termine (1991-2003) eseguito sul daino (*Dama dama*) nel lek della Tenuta Presidenziale di Castelporziano, Roma, Italia, dove i dati sono stati raccolti tramite radio-tracking e osservazioni dirette. Inoltre presento l'analisi di una serie temporale di 100 anni (1886-2003) relativamente all'abbondanza di tre specie sim-patriche di ungulati selvatici (cervi, caprioli e cinghiali).

Sono stati riportati nuovi risultati sull'ecologia comportamentale dei daini, con particolare riguardo alle strategie di successo riproduttivo e sulla metodologia statistica.

In primo luogo, ho dimostrato che, in accordo con la "scelta femminile" e la "dominanza maschile" nei daini, la letteratura presenta risultati contrastanti. Questa variabilità può riflettere differenze effettive tra le popolazioni studiate, ma può anche essere generata da differenze metodologiche e da deficit statistici nell'analisi dei dati. Ho quindi effettuato una revisione dei metodi statistici utilizzati finora negli studi in lek che mostrano una prevalenza di modelli lineari (LM) e modelli lineari generalizzati (GLM) che possono essere influenzati da problemi derivanti da come sono analizzati i rapporti causa-effetto; dalla multi-collinearity tra variabili esplicative e l’errata gestione delle distribuzioni non normali e non continue della variabili esplicative. Ho usato un set di dati di daino, per contrastare i metodi e le procedure impiegate finora, e propongo un nuovo approccio basato su modelli generalizzati di equazione strutturale (GSEMs). GSEM unisce il potere e la flessibilità di SEM e GLM in un modello unico. Abbiamo mostrato che i LM non riescono a individuare importanti covariate del successo copulatorio e produce stime molto imprecise. Minime variazioni nella trasformazione dei dati producono ampie variazioni nei risultati e il metodo sembra inaffidabile. I GLM migliorarono l'analisi, ma gli GSEM fornirono risultati migliori, in quanto l'utilizzo di variabili latenti riduce l'impatto degli errori di misura. Utilizzando GSEM, siamo riusciti a verificare ipotesi contrastanti e calcolare sia gli effetti diretti che indiretti e abbiamo raggiunto un'elevata precisione delle stime, che implica un'alta capacità predittiva.

In secondo luogo, ho mostrato che le femmine di daino meno esperte (giovani) incorrono in costi di viaggio più elevati, adottano forme indirette di selezione del maschio più spesso delle femmine adulta o delle femmine che permangono vicino al Lek. In particolare, le femmine più giovani sono rimaste più a lungo nel lek e in prossimità del lek rispetto a quelle adulte e sono tornate al lek dopo l’accoppiamento. Tuttavia, nonostante il tempo trascorso al lek, le

femmine giovani non sono in grado di selezionare i maschi di rango più elevato e si basano sulla scelta del territorio più che le adulte. Le femmine più lontane hanno visitato meno frequentemente il lek e sono arrivate dopo le femmine vicine. Sorprendentemente, non abbiamo trovato una copying tra le giovani e le adulte. I nostri risultati possono contribuire a chiarire la coevoluzione delle strategie di accoppiamento di entrambi i sessi degli ungulati nel lek.

In terzo luogo, ho mostrato che nelle comunità di ungulati delle aree mediterranee, esposte a fluttuazioni relativamente miti del clima, le interazioni trofiche come la dipendenza dalla densità, la concorrenza e la facilitazione hanno effetti più forti dei fattori ambientali come il clima. I risultati di tutte le analisi hanno confermato che la concorrenza intra-specifica è stata un elemento fondamentale per regolare i tassi di crescita all'interno della comunità di ungulati di Castelporziano, in linea con l'ipotesi secondo cui la regolazione indipendente dalla densità è più importante in quelle popolazioni dalle forti fluttuazioni climatiche.

L'importanza di questo studio risiede nel fatto che, a nostra conoscenza, è il primo studio comparativo dei modelli SEM e GSEM. In particolare, la possibilità di utilizzare i SEM per testare ipotesi in concorrenza e indagare sia gli effetti remoti che quelli prossimi è di particolare interesse per gli studi ecologici ed evolutivi. Una seconda constatazione originale di questa tesi è che la nostra conoscenza è il primo studio che si occupa del successo copulatorio da parte delle femmine di daino sia adulte che subadulte che formano lek.

Infine, ho proposto un nuovo approccio statistico basato sui modelli (SEMs) e (GSEMs) per la sperimentazione degli effetti dei fattori climatici e ambientali, della densità di popolazioni in competizione e della dipendenza dalla densità sul tasso di crescita dei cinghiali, dei daini e dei caprioli per verificare l'ipotesi che la dipendenza dalla densità e la competizione siano più rilevanti dei fattori climatici e ambientali nella regolazione delle popolazioni di ungulati del Mediterraneo, a differenza delle popolazioni che vivono in ambienti con clima più rigido e soggetto a maggiori fluttuazioni.

INTRODUCTION

Sexual selection is a fundamental evolutionary force that operates either through (i) direct competition between males or (ii) female mate choice which leads to the evolution of forms of exaggerated and useless ornaments in males (e.g. the peacock’s tail). The ornaments are supposed to display male genetic quality or the absence of sexually transmissible diseases (Davies et al. 2012). Albeit a long record of studies since Darwin’s time have addressed this problem, many questions about sexual selection remain open, and this continues to be a major research theme.

Sexual selection attains extreme levels in lek breeding where many males and females congregate in small aresa for mating, a situation that leads to fierce competition. In leks, males aggregate and defend small display territories located very close to one another.

This reproductive system is characterised by a strong asymmetry in male reproductive success (Wiley 1991; Alatalo et al. 1992; Höglund and Alatalo 1995; Alberts et al, 2003) that, in the absence of sexual coercion, is determined by a consensus of female choice.

For the present contribution, the main question is how to investigate the factors affecting male copulatory success in lek mating. In lekking species, the two sexes interact mainly during the rut (Wiley 1991; Höglund & Alatalo 1995) when males defend small display territories inside an arena or lek. For males, lekking is a high cost – high benefits strategy, in which the risk of injuries and even death is high, but a few dominant males may monopolize most of the copulations (Bradbury 1981; Apollonio et al. 1992). On the other hand, females are supposed to benefit from visiting a lek, since they can choose among several potential partners (Bradbury et al. 1985; Clutton-Brock et al. 1993).

Lekking has been described in many different taxa (reviewed by Hoglund & Alatalo 1995) such as insects, fishes, amphibians, reptiles, birds (Hoglund & Alatalo 1995 Rintamaki et al. 2001; Sardell et al. 2014; Kervinen et al. 2012) and mammals (Wiley 1991; Clutton-Brock et al. 1993; Clutton-Brock et al. 1988). In leks, male mating success is highly skewed (Fiske et al. 1998). However this features is not unique to leks and it is found in other reproductive systems as well (Lukas & Clutton-Brock 2014).

As a specific example, in fallow deer (*Dama dama*) (Clutton-Brock et al. 1988; Apollonio et al. 1989), the breeding system is highly variable and lekking is not the only strategy (Apollonio et al. 1992; Thirgood 1990). Independtly from the breeding system in this species the skew of male copulatory success appear always very high. Two main hypotheses have been proposed to explain the observed asymmetry in copulatory success: female choice, FCH, and male dominance, MDH, (Mackenzie et al. 1995; Kokko et al. 2003; Ryder et al. 2009). FCH (Clutton-Brock & Hasegawa 1989) assumes that the females select mates on the basis of the phenotypic traits of males, while according to MDH the copulatory success is determined by lek attendance and a high dominance rank (McElligott et al. 1998). In fallow deer, several studies pointed out that female choice is the most likely determinant of copulatory skew (Thirgood 1990; Ciuti et al. 2008; Clutton-Brock & McAuliffe 2009; Apollonio et al. 2014). However, Clutton-Brock et al. (1988) argue that copulatory success may not be solely related to female preferences for specific male traits, but it may also arise from different reasons, such as the need to minimize the risk of predation or harassment. Other authors, on the contrary, suggested that copulatory success strictly depends on male dominance rank (Apollonio et al. 1992; Say et al. 2003; Vannoni & McElligott 2008; Farrell et al. 2011; Jennings et al. 2012; Pitcher et al. 2014).

A number of different statistical techniques have been used to investigate the copulatory success in lekking species (e.g. Fiske et al. 1998). Most papers have applied standard linear models (e.g., Fiske et al. 1998; Kokko et al. 1998; McElligott et al. 1999), mixed models to account for repeated observations (e.g. Fričová et al. 2008; Bro-Jørgensen 2011a), or Generalized Linear Models to manage non-normal distributions. Finally, a few papers have used different approaches, such as logistic regression (Bro-Jørgensen 2008), path analysis (Focardi & Tinelli 1996b) and partial correlations (McElligott et al. 2001). A detailed list of the methods used in the literature is reported in S2 Table. A critical reading of this literature puts into light several methodological shortcomings: i) multicollinearity among explanatory variables (McElligott et al. 2001), (ii) erroneous handling of non-normal and non-continuous distributions of the response variable, and (iii) problems in inferring cause-effect relationships, so that no firm decision on the prevalence of female choice or male dominance could be established (Focardi & Tinelli 1996b).

Multicollinearity, which occurs when two or more predictors in a multiple regression model are highly correlated, leads to variance inflation and increase type-I errors, thus making some of the coefficients appear significant when they are not (Zuur et al. 2010).

Another important source of bias depends on erroneous handling of non-normal and non-continuous distributions of the response variable. Copulatory success is a classic example of such a variable; in leks, only a few males have access to mating, and this process leads to a zero-inflated distribution of copulations. In many cases, this problem is dealt with using square root or logarithm transformations (Fiske et al. 1998; Bro-Jørgensen 2008; McElligott et al. 2001; Ciuti et al. 2011), but despite this procedure being recommended in general biometry textbooks (e.g., Sokal & Rohlf 1995) its validity is restricted to cases when deviations from normality are only to limited extent. Moreover, discrete response variables containing many zeros cannot be transformed into normal distributions, and inference is doomed to be severely biased (O'Hara & Kotze 2010; Zuur et al. 2012).

There are concerns related to the link between correlations and causation, which are tricky to deal with. Explanatory variables and copulatory success may, in fact, appear unrelated when they are related, or on the contrary, they may be correlated even when no causal link is present. A spurious or missing correlation may arise for several reasons which include (i) a common causation that induces a false relationship or cancels out an existing association, (ii) a reciprocal association loop, (iii) a conditional relationship between explanatory and response variables following the value of a third control variable, or (iv) a non-linear association between dependent and independent variables (Shipley 2000; Navidi 2006; McDonald 2014; Kendall 2015) When a correlation between two variables is detected, cause-effect relationships cannot be easily deduced without further assumptions (Shipley 1999; Shipley 2000). The best way to test causal relationships is to use a proper experimental design where the hypothetical cause is directly manipulated (Shipley 1999). However, manipulative experiments are difficult to achieve, and researchers have to rely mainly on observational studies (Höglund & Alatalo 1995; Fiske et al. 1998; Jiguet & Bretagnolle 2006).

The problem of inferring cause-effect relationships among variables can be addressed by path analysis or Structural Equation Models (SEM) (Pearl et al. 2016). In field studies often the variables of interest cannot be directly recorded by the observers. For instance, we cannot measure the "sex appeal" of males (Sih et al. 2002). However, we can measure some traits we expect to be correlated to "sex appeal" and so obtain an indirect evaluation of the variable of interest. This is the same done in principal component analysis: a reduced number of meaningful factors are estimated from the correlations among a large number of descriptors. In SEM terminology, we refer to the unobservable factors as latent and to the observed descriptors as manifest. A SEM is a combination of a measurement model that defines latent variables using one or more manifest variables and a structural model that imputes causal relationships between latent variables (Shipley 2000). The development of a measurement model is also important to control for the errors introduced during observations, i.e., it represents a state space model for the unobserved variables of interest. In this way, a latent variable is not directly observed, but its existence is inferred by the way it influences manifest variables that can be directly observed (Shipley 2000).

One known limitation of standard SEM is to assume that all variables are normally distributed (Grace et al. 2012). The introduction of Generalized Structural Equations Models (GSEM), may overcome this limitation. In GSEM, it is possible to have a model with both continuous and discrete variables grouped together in the same latent construct. As such, GSEM combines the power and flexibility of both SEM and GLM in a unified modeling framework. The advantages of GSEM are: (i) to evaluate potential causal relationships with the "structural model"; (ii) to consider both direct and indirect effects of multiple interacting factors, simultaneously (Shipley 2000; Pearl et al. 2016; Agresti 1990; Muthén B, Asparouhov 2015); (iii) the possibility of using appropriate probability density functions other than the normal one for manifest indicators and latent constructs.

In **Chapter 1**, we contrast the main statistical methods used in literature to GSEM using data from a specific study case about fallow deer lekking behaviour. First, we reviewed the available literature on lekking behaviour to obtain an overview of the statistical methods used. Secondly, we fitted the main types of models used. Third, within a SEM framework, we formulated two models, one describing the FCH and the other the MDH hypotheses, and fitted them using both SEM and GSEM, for comparison. Finally, we compared the predictive performances of the different methods using information theoretic indexes (AIC and BIC), residual analysis, and precision of regression coefficients.

We analyze the deer population from the point of view of females. There is consensus that the lekking population of Castelporziano Preserve operates a female choice. In leks, females do not get any resources from males except genes, even if some authors have argued that females may obtain direct benefits, such as the reduction of transmission of venereal diseases and ectoparasites, or the reduction of social interference (Bradbury 1981; Clutton-Brock et al. 1993; Höglund & Alatalo 1995; Apollonio et al. 2014). The ability to compare many males on the same stage leads females to visit these aggregations (Bradbury, 1981). Female choice seems to play an important role in the evolution of the lek and, for the understanding of these processes, it is essential to identify which are the costs and benefits for females (Wrangham 1980; Pomiankowski 1987; Reynolds & Gross, 1990; Clutton-Brock et al. 1993).

A small number of ungulates are known to form leks such as the topi (*Damaliscus lunatus,* Bro-Jørgensen 2002); the sika deer (*Cervus nippon*, Bartoš et al. 2003), the Uganda kob (*Kobus kob thomasi*, Deutsch 1994), the white-eared kob (*K. k. leucotis*, Fryxell 1987) and the Kafue lechwe (*K. leche kafuensis*, Nefdt 1995). The fallow deer (*Dama dama*) is an ideal species to study lek mating given that this cervid forms large aggregations, individual behaviour can be easily recorded and males are easily identifiable by their palmed antlers in the absence of tags. Most of previous field studies concerning leks of fallow deer focused on the covariates of male reproductive success (see Lombardi et al. 2017 and references therein), while there is much less information on female tactics of mate choice (Clutton-Brock et al. 1989; Apollonio et al. 2014; for non-lekking populations and controlled experiments see also McComb & Clutton-Brock 1994; Komers et al. 1999; Farrell et al. 2011; Briefer et al. 2013; Naulty et al. 2013). Our paper aims to fill this gap of knowledge using the availability of information on the behaviour of a number of individually-marked females in the lek of Castelporziano (Italy).

In particular, in **Chapter 2** we verify whether or not variable reproductive costs and/or experience of does are associated with different tactics of mate choice.

High costs for lekking females can be expected because of (i) increased energetic expenditure to get to the lek; (ii) increased predation (or accident) risks during the displacement to and from the lek; (iii) less time left for other activities, such as foraging (Gibson & Bachman 1992). In this study we overlook the analysis of predation risk because large predators were absent in the study area at the time of the study. Energy expenditure and accident risks are plausibly correlated to the distance between one female's home range and the lek, while the permanence in the lek (when located outside a female's home range) is probably correlated to the cost of missed opportunities.

We can safely assume that female experience improves with age only if a doe mates in the lek for several consecutive years. Accordingly, we investigated female mate tactics in relation to age.

As stated above, an important motivation for a female to visit a lek is the possibility of choosing an appropriate mate from among a large number of suitors. A female makes a direct assessment when, after having visited all or most males, chooses one of them exclusively on the basis of his phenotype (Janetos 1980; Andersson & Simmons 2006). Although this behaviour can be quite effective, it requires considerable effort and discerning skills by females. On the contrary, secondary tactics of mate assessment occur when a female choses a partner using cues other than male phenotypic traits. This approach, described in several lekking species (Gibson & Höglund 1992; Fiske et al. 1996; Kokko et al. 1999), may allow for an efficient choice at reduced costs. Importantly, secondary tactics have the potential to increase the asymmetry observed in male reproductive success (Wade & Pruett-Jones 1990; Alonzo 2008).

In this thesis we want to assess whether females are more likely to make use of secondary strategies in relation to both the location of their home range with respect to the lek (displacement costs) and their age (experience).

We investigated three secondary tactics:

1. *Aggregation*. Clutton-Brock *et al*. (1989) found that the number of females increases in territories where a harem is already present: females could aggregate in order to have higher probabilities to join a successful male.
2. *Copying*. A female mates with a male that was previously observed to mate with other females (Bradbury & Gibson 1983);
3. T*erritory choice.* A female chooses a male that is defending a territory where other copulations have taken place earlier (Gibson 1992).

A main difference between *copying* and *territory choice* is that the latter can be based also on indirect cues, such as pheromones or territorial marks, while copying relies only on the direct observation of copulations. *Aggregation* differs from *copying* because it may allow females to have a quick idea about the position of successful males in the lek, even in absence of observed copulations.

We formulated a set of six (not mutually exclusive) hypotheses: H1-Females with no or reduced travel costs should adopt more often a direct assessment tactic than females living far away. We therefore expect that a) the time spent at lek, the number of female visits to the lek, and the date of arrival are inversely dependent on the distance between a female home range and the lek. More specifically, we predict that females using a direct choice tactic are present in the lek before the onset of the oestrus in order to observe displaying bucks and evaluate their quality. Consequently, we also expect these females (b) to visit a higher number of territories and to spend more time in the vicinity of bucks.

H2-Secondary tactics allow reduced mating costs for females (Fiske *et al.* 1996; Kokko et al. 1999). We thus expect that females with higher travel costs would be prone to use secondary mating tactics and, more specifically, we expect that these females are more likely to be (a) observed within female clusters (*aggregation*), and/or (b) to mate with a buck that copulated immediately before her own copulation (*copying*) or (c) mating in a territory where more copulations had occurred earlier (*territory choice*).

H3-Secondary strategies could be used by less experienced (younger) females that are not able to make a good choice of their own. In this case, predictions H2a-2c should be confirmed in young females independent of the distance of their home range from the lek (H3a-3c). We also expect that younger females (d) would stay in the lek longer than adult ones to observe mating behaviour and, consequently, (e) to spend more time in territories and in the vicinity of bucks. This makes sense if they visit the lek when the mating activity is well developed and so we do not expect (f) that they arrive at the lek before adult females.

H4-Females using *copying* and *territory choice* (a) should mate later than the ones that make a direct assessment and, consequently, (b) we expect that the asymmetry in male mating success increases over the course of the mating season.

H5-Females do not need to spend any time at lek after mating, except if this is useful for gaining experience. Thus we expect that young females stay longer in the lek than adult ones after their own mating, irrespective of the distance lek-home range.

H6-Secondary tactics could increase the precision in mate assessment (Balmford 1991; Gibson & Höglund 1992). If this holds, females that follow these tactics should make a better choice than the ones that make a direct assessment. We thus expect (a) highly successful males to be more likely to mate with females that adopt a secondary tactic. We are allowed to use the total mating success of a buck as a proxy for its quality, since Losey et al. (1986) theoretically demonstrated that at least 40% of females should adopt a direct choice behaviour, and that direct choice is, on average correct, in order for copying to be an evolutionary stable strategy. Alternatively (b) we can hypothesize that females that make a direct assessment then mate with males that are, on average, of higher quality which results in a high-cost but high-precision female tactic.

Finally, the thesis presents a study of the Ungulates population, including fallow deer, in the Mediterranean environment. Population ecology is the study of how populations change over time and space. The ability to predict population change is fundamental in many different cotexts, and it is the basis both for a sound management of natural populations. Many important questions in ecology and evolutionary biology can only be answered with data that extend over several decades and answering a substantial proportion of questions requires records of the life histories of popultion. Among the factors recognised as important in affecting population changes, climate and competition play a major role (Putman 2012). Climate is an important determinant of many ecological processes (Stenset et al. 2002), and both direct and indirect influences on the dynamics of ungulate populations have been described (reviews in Weladji et al. 2002; Mysterud et al. 2003). In North Europe, for example, ungulate population are known to decline after particularly snowy winters (e.g. Jacobson et al. 2004; Grøtan et al. 2005; Mysterud and Østbye 2006b), and negative effects of severe winter conditions on individual body mass (Cederlund et al. 1991) have been described as well. Climate conditions (temperature and precipitation) during early summer, whose effects are mediated by plant growth and availability, are important determinants of individual growth (Langvatn et al. 1996; Mysterud et al. 2001). As all ungulate species (e.g. *deer)*, have a fairly fixed breeding time, changes in environmental conditions may determine a mistmatch between individual needs and food supply, with negative effects on population growth rates (e.g. Plard et al. 2014).

According to Putman (1996) competition is expected to be the most important type of interaction among large herbivores in the absence of large predators. The most frequent type of competition among large herbivores is exploitation competition (Dolman & Wäber 2008), which occurs when the use of a resource by one individual reduces the availability of that resource to another individual. Many studies have suggested that, when resources are limited, the potential for competition is high among sympatric species, as their habitats and nutritional niches often overlap (Bartoš et al. 2002). Examples are provided by Bartoš et al. (2002) for white-tailed deer Odocoileus virginianus, fallow deer *Dama dama*, red deer *Cervus elaphus*, and roe deer *Capreolus capreolus*; Focardi et al. (2006) for roe deer and fallow deer; Hemami et al. (2004) for roe deer and muntjac *Muntiacus reevesi*; Mysterud et al. (2007) for roe deer and wild boar *Sus scrofa*; Storms et al. (2008) for red deer *Cervus elaphus*, and roe deer *Capreolus capreolus*; Richard et al. (2010) for red deer *Cervus elaphus* and roe deer; Ferretti et al. (2012) for roe deer and fallow deer; Imperio et al. (2012) for roe deer *Capreolus capreolus italicus*, red deer, fallow deer and wild boar *Sus scrofa majori*.

Chapter 3, address a different topic and aims to elucidate which factors drive population fluctuations, in a guild of Mediterranean ungulates. In particular, I re-analysed the population dynamics of three wild ungulate species in the Castelporziano Preserve using the methodological framework for causal analysis described in Chapter 1 (Lombardi et al. 2017). I considered three species : the Italian roe deer *Capreolus capreolus italicus*, the Maremma wild boar *Sus scrofa majori* (both endemic to Italy), and the alien fallow deer *Dama dama*. The native red deer *Cervus elaphus*, is not present today in the Preserve, as it was completely eradicated during WWII (Imperio et al. 2012). Information on the temporal changes of the three species were obtained from detailed bag counts from hunting drives during the period 1878–1986. It is known that competition plays an important role in regulating the abundance of these species in Mediterranean environmnets. As for example, Focardi et al. (2006), demonstrated that at Castelporziano (Rome, Italy) high densities of fallow deer may reduce habitat quality for roe deer, forcing the latter to achieve smaller body size and larger home ranges. In another area (Maremma Park, Tuscany, Italy), Ferretti et al. (2008, 2011a) documented behavioural interference of fallow to roe deer, showing that fallow deer is able to exclude the roe deer from feeding sites,also using direct aggression (Ferretti 2011). In both cases, roe deer numbers declined as fallow deer density increased (Ferretti et al. 2011a; Focardi et al. 2006). It is not fully known, however, if climate forcing may also play a role in the dynamics of these species. The aim of this study is to detect which factors (endogenous and/or exogenous) affect the per capita growth rate, r, and thus drive population fluctuations in these species. Two main hypotheses have been proposed and compared: i) the complexity of the ungulate community influences intra- and inter-specific interactions; ii) in Mediterranean environments intra- and inter-specific interactions are stronger than climate forcing.

List of scientific research products

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CHAPTER 1: *Generalized structural equations improve sexual selection analyses*

Sexual selection is an intense evolutionary force, which operates through competition for the access to breeding resources. There are many cases where male copulatory success is highly asymmetric, and few males are able to sire most females. Two main hypotheses were proposed to explain this asymmetry: "female choice" and "male dominance". The literature reports contrasting results. This variability may reflect actual differences among studied populations, but it may also be generated by methodological differences and statistical shortcomings in data analysis. A review of the statistical methods used so far in lek studies, shows a prevalence of Linear Models (LM) and Generalized Linear Models (GLM) which may be affected by problems in inferring cause-effect relationships; multi-collinearity among explanatory variables and erroneous handling of non-normal and non-continuous distributions of the response variable. In lek breeding, selective pressure is maximal, because large numbers of males and females congregate in small arenas. We used a dataset on lekking fallow deer (*Dama dama*), to contrast the methods and procedures employed so far, and we propose a novel approach based on Generalized Structural Equations Models (GSEMs). GSEMs combine the power and flexibility of both SEM and GLM in a unified modeling framework. We showed that LMs fail to identify several important predictors of male copulatory success and yields very imprecise parameter estimates. Minor variations in data transformation yield wide changes in results and the method appears unreliable. GLMs improved the analysis, but GSEMs provided better results, because the use of latent variables decreases the impact of measurement errors. Using GSEMs, we were able to test contrasting hypotheses and calculate both direct and indirect effects, and we reached a high precision of the estimates, which implies a high predictive ability. In synthesis, we recommend the use of GSEMs in studies on lekking behaviour, and we provide guidelines to implement these models.

Introduction

Sexual selection is a fundamental evolutionary force that operates either through (i) direct competition between males or (ii) female mate choice which leads to the evolution of forms of exaggerated and useless ornaments in males (e.g. the peacock's tail). The ornaments are supposed to display male genetic quality or the absence of sexually transmissible diseases (Davies et al. 2012). Albeit a long record of studies since Darwin’s time have addressed this problem, many questions about sexual selection remain open, and this continues to be a major research theme. For the present contribution, the main question is how to investigate the factors affecting male copulatory success in lek mating. In lekking species, the two sexes interact mainly during the rut (Wilwy 1991; Höglund & Alatalo 1995) when males defend small display territories inside an arena or lek. For males, lekking is a high cost – high benefits strategy, in which the risk of injuries and even death is high, but a few dominant males may monopolize most of the copulations (Bradbury 1981; Apollonio et al. 1992). On the other hand, females are supposed to benefit from visiting a lek, since they can choose among several potential partners (Bradbury et al. 1985; Clutton-Brock et al. 1993).

Lekking has been described in many different taxa (reviewed by Hoglund & Alatalo 1995) such as insects, fishes, amphibians, reptiles, birds (Hoglund & Alatalo 1995 Rintamaki et al. 2001; Sardell et al. 2014; Kervinen et al. 2012) and mammals (Wiley 1991; Clutton-Brock et al. 1993; Clutton-Brock et al. 1988). In leks, male mating success is highly skewed (Fiske et al. 1998). However this features is not unique to leks and it is found in other reproductive systems as well (Lukas & Clutton-Brock 2014).

As a specific example, in fallow deer (*Dama dama*) (Clutton-Brock et al. 1988; Apollonio et al. 1989), the breeding system is highly variable and lekking is not the only strategy (Apollonio et al. 1992; Thirgood 1990). Independtly from the breeding system in this species the skew of male copulatory success appear always very high. Two main hypotheses have been proposed to explain the observed asymmetry in copulatory success: female choice, FCH, and male dominance, MDH, (Mackenzie et al. 1995; Kokko et al. 2003; Ryder et al. 2009). FCH (Clutton-Brock & Hasegawa 1989) assumes that the females select mates on the basis of the phenotypic traits of males, while according to MDH the copulatory success is determined by lek attendance and a high dominance rank (McElligott et al. 1998). In fallow deer, several studies pointed out that female choice is the most likely determinant of copulatory skew (Thirgood 1990; Ciuti et al. 2008;, Clutton-Brock & McAuliffe 2009; Apollonio et al. 2014). However, Clutton-Brock et al. (1988) argue that copulatory success may not be solely related to female preferences for specific male traits, but it may also arise from different reasons, such as the need to minimize the risk of predation or harassment. Other authors, on the contrary, suggested that copulatory success strictly depends on male dominance rank (Apollonio et al. 1992; Say et al. 2003; Vannoni & McElligott 2008; Farrell et al. 2011; Jennings et al. 2012; Pitcher et al. 2014).

A number of different statistical techniques have been used to investigate the copulatory success in lekking species (e.g. Fiske et al. 1998). Most papers have applied standard linear models (e.g., Fiske et al. 1998; Kokko et al. 1998; McElligott et al. 1999), mixed models to account for repeated observations (e.g. Fričová et al. 2008; Bro-Jørgensen 2011a), or Generalized Linear Models to manage non-normal distributions. Finally, a few papers have used different approaches, such as logistic regression (Bro-Jørgensen 2008), path analysis (Focardi & Tinelli 1996b) and partial correlations (McElligott et al. 2001). A detailed list of the methods used in the literature is reported in S2 Table. A critical reading of this literature puts into light several methodological shortcomings: i) multicollinearity among explanatory variables (McElligott et al. 2001), (ii) erroneous handling of non-normal and non-continuous distributions of the response variable, and (iii) problems in inferring cause-effect relationships, so that no firm decision on the prevalence of female choice or male dominance could be established (Focardi & Tinelli 1996b).

Multicollinearity, which occurs when two or more predictors in a multiple regression model are highly correlated, leads to variance inflation and increase type-I errors, thus making some of the coefficients appear significant when they are not (Zuur et al. 2010).

Another important source of bias depends on erroneous handling of non-normal and non-continuous distributions of the response variable. Copulatory success is a classic example of such a variable; in leks, only a few males have access to mating, and this process leads to a zero-inflated distribution of copulations. In many cases, this problem is dealt with using square root or logarithm transformations (Fiske et al. 1998; Bro-Jørgensen 2008; McElligott et al. 2001; Ciuti et al. 2011), but despite this procedure being recommended in general biometry textbooks (e.g., Sokal & Rohlf 1995) its validity is restricted to cases when deviations from normality are only to limited extent. Moreover, discrete response variables containing many zeros cannot be transformed into normal distributions, and inference is doomed to be severely biased (O'Hara & Kotze 2010; Zuur et al. 2012).

There are concerns related to the link between correlations and causation, which are tricky to deal with. Explanatory variables and copulatory success may, in fact, appear unrelated when they are related, or on the contrary, they may be correlated even when no causal link is present. A spurious or missing correlation may arise for several reasons which include (i) a common causation that induces a false relationship or cancels out an existing association, (ii) a reciprocal association loop, (iii) a conditional relationship between explanatory and response variables following the value of a third control variable, or (iv) a non-linear association between dependent and independent variables (Shipley 2000; Navidi 2006; McDonald 2014; Kendall 2015) When a correlation between two variables is detected, cause-effect relationships cannot be easily deduced without further assumptions (Shipley 1999; Shipley 2000). The best way to test causal relationships is to use a proper experimental design where the hypothetical cause is directly manipulated (Shipley 1999). However, manipulative experiments are difficult to achieve, and researchers have to rely mainly on observational studies (Höglund & Alatalo 1995; Fiske et al. 1998; Jiguet & Bretagnolle 2006).

The problem of inferring cause-effect relationships among variables can be addressed by path analysis or Structural Equation Models (SEM) (Pearl et al. 2016). In field studies often the variables of interest cannot be directly recorded by the observers. For instance, we cannot measure the "sex appeal" of males (Sih et al. 2002). However, we can measure some traits we expect to be correlated to "sex appeal" and so obtain an indirect evaluation of the variable of interest. This is the same done in principal component analysis: a reduced number of meaningful factors are estimated from the correlations among a large number of descriptors. In SEM terminology, we refer to the unobservable factors as latent and to the observed descriptors as manifest (a detailed discussion is presented in S3 Text and in S3 Fig). A SEM is a combination of a measurement model that defines latent variables using one or more manifest variables and a structural model that imputes causal relationships between latent variables (Shipley 2000). The development of a measurement model is also important to control for the errors introduced during observations, i.e., it represents a state space model for the unobserved variables of interest. In this way, a latent variable is not directly observed, but its existence is inferred by the way it influences manifest variables that can be directly observed (Shipley 2000).

One known limitation of standard SEM is to assume that all variables are normally distributed (Grace et al. 2012). The introduction of Generalized Structural Equations Models (GSEM), may overcome this limitation. In GSEM, it is possible to have a model with both continuous and discrete variables grouped together in the same latent construct. As such, GSEM combines the power and flexibility of both SEM and GLM in a unified modeling framework. The advantages of GSEM are: (i) to evaluate potential causal relationships with the "structural model"; (ii) to consider both direct and indirect effects of multiple interacting factors, simultaneously (Shipley 2000;

Pearl et al. 2016; Agresti 1990; Muthén B, Asparouhov 2015); (iii) the possibility of using appropriate probability density functions other than the normal one for manifest indicators and latent constructs.

In this paper, we contrast the main statistical methods used in literature to GSEM using data from a specific study case about fallow deer lekking behaviour. First, we reviewed the available literature on lekking behaviour to obtain an overview of the statistical methods used. Secondly, we fitted the main types of models used. Third, within a SEM framework, we formulated two models, one describing the FCH and the other the MDH hypotheses, and fitted them using both SEM and GSEM, for comparison. Finally, we compared the predictive performances of the different methods using information theoretic indexes (AIC and BIC), residual analysis, and precision of regression coefficients.

Materials and Methods

Study area and data collection

Field observations were carried out during 1991 and 1992 ruts (September-October) in the Preserve of Castelporziano near Rome (Italy) (coordinate), an fenced area covering 42 km^2. The habitat is characterized by an old-growth natural oak wood, with both evergreen *(Quercus ilex* and *Q. suber)* and deciduous (mainly *Q. cerris* and *Q. frainetto*) tree species. A detailed description of the vegetation of the study area can be found in Bianco et al. 2001. Information on ungulate populations are given in Focardi et al. 2015 and Imperio et al. 2012. The dataset was used to estimate two different dominance indexes: (a) *Dom* (Clutton-Brock et al. 1979); (b) David's score, *Ds* (Gammel et al. 2003). To obtain index values comparable across years, *Dom* and *Ds* were relativized to the number of fights observed in each year. The number of observed copulations achieved by a buck in one rut was used as a measure of copulatory success (*CopS*).

Two measures of lek attendance were computed: LA_1, is the number of total days in which an animal was seen at the lek and LA_2 is the number of days the animal was able to hold a territory. Finally, we estimated the average number of females observed in one buck's territory (harem size - *HS*) and courtship success (*CourtS*) as the number of courtships terminated with a copulation divided by the total number of attempts (number of copulations /number of courtship events, for every male).

Two variables were used: a) the total number of spellers (*TotS*) and b) a measure of fluctuating asymmetry for small spellers (Møller 1990; Møller 1996) ASS_T.

Further details on study area, data collection, data validation and measures computations are provided in S1 Text, S1 Table, S1 Fig and S2 Fig in Supporting information.

Ethic Statement

This work does not imply animal handling or capture. The "Segretariato alla Presidenza della Repubblica" was the authority responsible for the permission to work in the Preserve of Castelporziano, Rome, (Italy). The fieldwork was based on a research and management agreement between the I.S.P.R.A -The Italian National Institute for Environmental Protection and Research (ex I.N.F.S. National Institute for Wildlife) (former institution of SF 1988-2011), the Director of the Preserve of Castelporziano, Dr. A. Demichelis, the Preserve research responsible, Dr. A. Tinelli, in collaboration with the Presidential Estate rangers, and the Corpo Forestale dello Stato (C.F.S.) under the combined prescriptions of the Italian law which regulates studies on wild species and does not require that the I.S.P.R.A. obtain permits from any other authorities. The field study did not involve endangered or protected species and this implied that it was not required any approval from Institutional Animal Care and Use Committee. The study was not carried out on private land.

Statistical analysis

We compared several modelling approaches described in the literature. We were aware that some of these approaches are inherently flawed, but we decided to use them due to their widespread use in the pertinent literature on leks (cfr. S2 Text and S2 Table). All the tested models have CopS as the response variable. Note that CopS is discrete by definition (because it is a count) and hence cannot be assumed to be normally distributed.

Linear Models and Generalized Linear Models

The copulatory success of the *i*-th buck (*CopS*) is modelled as:

$$CopS_i = \beta_0 + \beta_1 x_{1,i} + \beta_2 x_{2,i} + \ldots + \beta_p x_{p,i} + \varepsilon_{CopS} \qquad (Eq\ 1)$$

where the $x_{p,i}$ are predictor variables, the βs regression coefficients and ε_{CopS} is the error term.

Following the approaches described in the literature, we first used ordinary least squares regression where the response variable *CopS*. was untransformed, log-transformed, or square-root transformed. Secondly, we used GLMs for count data. The following models were considered:

- LM_1, multiple regression model without *CopS* transformation;
- LM_2 where the dependent variable is log(*CopS +1*);
- LM_3 where the dependent variable is log(*CopS +0.5*);
- LM_4 where the dependent variable is log(*CopS +0.1*);
- LM_5 where the dependent variable is $CopS^{0.5}$;
- GLM_1, Generalized Linear Model where *CopS* follows a Poisson distribution;
- GLM_2, assuming that *CopS* follows a Negative Binomial distribution;
- GLM_3, assuming that *CopS* follows a Zero Inflated Poisson distribution (ZIP);
- GLM_4, assuming that *CopS* follows a Zero Inflated Negative Binomial distribution (ZINB);
- GLM_5, assuming that *CopS* follows a Hurdle at Zero Distribution (Hurdle). In the Hurdle models a Bernoulli probability governs the binary outcome of whether a count variable has a zero or positive realization. When the realization is positive the conditional distribution is modelled by a truncated at zero count data model.

For each type of model we considered both the full model, which includes all significant ($P<0.05$) and non-significant coefficients and the Minimal Adequate Models (MAM) which include only significant values (Crawley 2002) MAMs, hereafter denoted by the suffix *r* (e.g. $GLM_{4,r}$) were obtained using a *p*-value selection procedure (Murthaug 2014).

Akaike information criterion (AIC) and Bayesian information criterion (BIC) were also computed to assess model performances.

Statistical analysis was carried out in R (R Core Team 2016) using the packages *fitdistrplus, gamlss, pscl, vcd*.

Generalized Structural Equation Models

A SEM requires the *a-priori* definition of links among model variables in the form of a regression equations system. The goal of this class of models is minimize the difference between estimates and expectations variance-covariance matrix of data.

Latent variables are unobserved factors denoted, $\eta_1, \eta_2, \ldots.., \eta_n$ that represent an hypothetical construct that can be inferred by the way it influences manifest or observed variables (continuous, $Y_i = y_1, y_2, .., y_n$) (Shipley 2000; Muthén et al. 2015).

A SEM model is composed by two sub-models: a measurement model that describes the relationships between latent variables and their manifest variables and a structural or causal model that constitutes a directional chain system that describes the hypothetical causal relationship between the constructs of theoretical interest (latent variables) using *path diagrams* (Fig 1a and 1b).

Structural coefficients or regression coefficient (γ, β, λ) represent the effects of each independent variable on the dependent variable (Fig 1a and 1b).

A manifest variable, in a SEM with latent variables, plays a role of endogenous variable if it is predicted by another variable in the model and is therefore a response variable; it is assumed to be generated as a linear function of its latent dimension and the residual error term represents the imprecision in the measurement process. An exogenous variable whose variation is not explained in a model (i.e. fluctuating asimmetry of small spellers ASS_T or *Dom*). A description of SEM modelling is reported in S3 Text, S3 Table and S3 Fig.

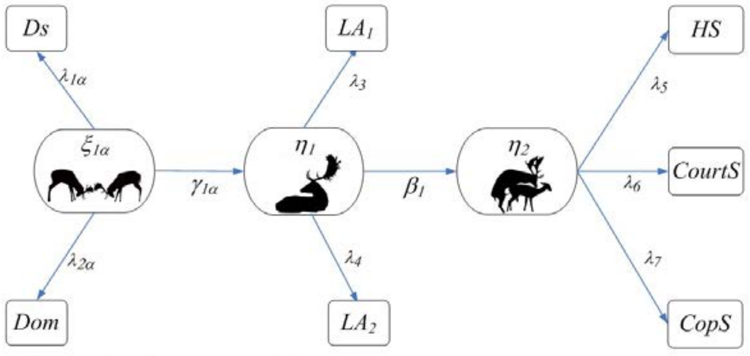
GSEMs represent a generalization of SEMs by allowing the use of discrete variables and non-Gaussian distributions. They combine observed (or manifest) and latent variables representing unmeasured constructs. A GSEM (Bollen & Pearl 2013) reads:

$$\boldsymbol{\eta} = f_\eta\,(\eta, \xi, \zeta)$$

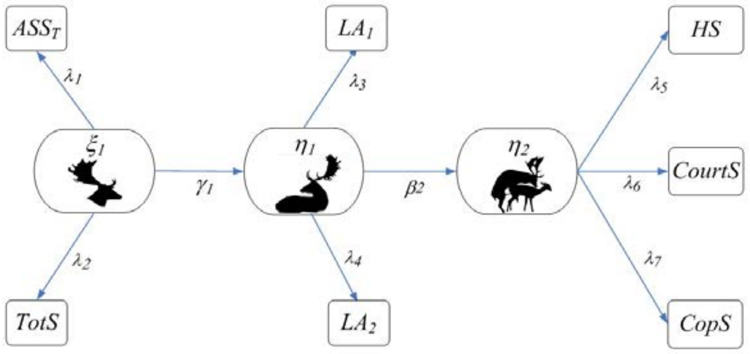
$$\boldsymbol{x} = f_x\,(\eta, \delta) \qquad \text{(Eqs 2)}$$

$$\boldsymbol{y} = f_y\,(\eta, \varepsilon)$$

where x and y are vectors of manifest variables and η, ξ, ζ represent the latent variables, while δ, and ε denote the error terms. The functions (f_η, f_y, f_x) provide a general way to represent the connections between the variables within the parentheses to those on the left hand side of each equation. We developed and compared two different causal models, one assuming that copulatory success is determined by MDH and the other one based on FCH.



a)Male dominance model



b) Female choice model

Fig 1. Path diagrams for a) the "dominance male" model (MDH) and b) "female choice" model (FCH). Variable names are: ASS_T = the fluctuating asymmetry of small antler's spellers*;* *TotS* = total number of small and large antler's spellers*;* *Dom* = Dominance Index (Clutton-Brock Index (Clutton-Brock et al. 1979)) divided by the total number of bucks of each year; *Ds* = the David's score (Gammel et al. 2003) divided for the total number of bucks of each year; LA_1=number of days in which the animal was present in the lek. LA_2= total number of days of presence/territory in different locations of the same lek. *HS* = average number of females in a male's territory; *CourtS* = the fraction of courtship events terminated with a copulation (number of copulations / number of courtship events, for every male); *CopS* = total copulatory success of the i-th buck in one rut. The number of observations is the same for all models (N=118). Symbols and variables are described in the text and in S1 Table.

We verified that the number of parameters is identifiable according to rules 1 and 3 of Shipley (2000). We used a robust maximum likelihood estimator and a sandwich estimator (Bollen & Pearl 2013). We fitted GSEMs with both Mplus (Muthén & Muthén 2015) and STATA (STATA StataCorp. Stata Statistical Software, 2015). We used both softwares to check that the results are identical. Further STATA provided case-specific residuals which are not outputted by Mplus. On the other hand, Mplus returns the standardized path coefficients and total, direct, and indirect effects which STATA does not compute. The STATA and Mplus codes used to generate SEM and GSEM models are presented in S4 Text.

Models' comparison

Unfortunately, there is no a simple method for comparing these different sets of models. GLMs and LMs can be compared by AIC or BIC, but only if the dependent variable is not transformed (Burnham & Anderson 2002). To overcome this problem and make all LMs and GLMs comparable, we calculated the maximum likelihood estimates from the log-transformed or root square–transformed model applying the formula reported in Weiss (Weiss 2016) (see S5 Text for details).

The comparison of SEM or GSEM with AIC is questionable due to the presence of latent variables which increase AIC values making these models not comparable to GLMs (Skrondal & Rabe-Hesketh 2004). On the other hand, the use of absolute fitting indexes is vulnerable to criticisms (Bentler & Bonett 1980; Hooper et al. 2008).

We compared models by two different approaches. First, we measured the precision of each estimated regression coefficient $\widehat{\beta}$ by computing its coefficient of variation ($CV = \frac{SE(\widehat{\beta})}{|\widehat{\beta}|} = \frac{1}{|\widehat{\beta}|/SE(\widehat{\beta})} = \frac{1}{|T|} = \sqrt{\chi_1^2}$, where T is the statistic test and χ_1^2 is the chi-square test with one degree of freedom). For a more general evaluation of the model's precision, we computed the median CV for the parameters estimated by each model (Lande 1977). Second, we performed an analysis of case-specific residuals. In principle, if a model correctly fits the data, the residuals are expected to have zero mean, normal distribution, without any pattern or structure. We visually checked residual distributions and computed their mean, variance, and kurtosis. The best distribution is the one with the smallest variance of residuals, symmetrical and centered around zero.

Definition of working hypotheses

In this paper, we contrast two working non-nested hypotheses, "male dominance" (MDH) and "female choice" (FCH). The structure of the models corresponding to the Male Dominance Hypothesis (MDH) and the Female Dominance Hypothesis (FDH) is shown in Fig 1. We have assumed, according to literature, the existence of four latent variables: ξ_1 represents the effect of antler shape and is described by ASS_T and *TotS*, ξ_{1a} represents male dominance and is described by *Dom* and *Ds*, η_1 represents lek attendance (LA_1 and LA_2). Finally, η_2 represents courtship and is measured by *HS*, *CourtS,* and *CopS*. The use of latent variables allowed us to reduce the unavoidable errors in the measurement of manifest variables. For MDH we assume that ξ_{1a} influences η_1, or in other words the fighting ability of bucks determines their lek attendance and territory holding. Being able to defend a territory allowed a buck to keep a harem and finally to sire females. For the FCH we assume that male phenotypic quality, ξ_1, which represents its health and physical fitness, allows the buck to stay in the lek for a long time and to be selected by wandering females.

Note that SEM allows us to study the effects of remote and proximate causes of male copulatory success in the same statistical framework. Further, the use of latent variables reduces the unavoidable errors in the measurement of manifest variables. Once the measurement model is defined, we can establish appropriate causal relationships among latent variables.

The MDH is implemented by the following system of regression equations (Fig 1a):

$$Ds = \lambda_{1a}\xi_{1a} + \delta_{Ds}$$

$$Dom = \lambda_{2a}\xi_{1a} + \delta_{Dom}$$

$$LA_1 = \lambda_3\eta_1 + \epsilon_{LA_1} \qquad \text{(Eqs 3)}$$

$$LA_2 = \lambda_4\eta_1 + \varepsilon_{LA_2}$$

$$HS = \lambda_5\eta_2 + \varepsilon_{HS}$$

$$CourtS = \lambda_6\eta_2 + \varepsilon_{CourtS}$$

$$CopS \sim Poisson(\mu), \log\big(\mu(CopS)\big) = \lambda_7\eta_{2.}$$

The model for FCH is represented in Fig 1b and reads:

$$ASS_T = \lambda_1 \xi_1 + \delta_{ASS_T}$$

$$TotS = \lambda_2 \xi_1 + \delta_{TotS}$$

$$LA_1 = \lambda_3 \eta_1 + \epsilon_{LA_1} \qquad \text{(Eqs 4)}$$

$$LA_2 = \lambda_4 \eta_1 + \varepsilon_{LA_2}$$

$$HS = \lambda_5 \eta_2 + \varepsilon_{HS}$$

$$CourtS = \lambda_6 \eta_2 + \varepsilon_{CourtS}$$

$$CopS \sim Poisson(\mu), \log\big(\mu(CopS)\big) = \lambda_7 \eta_2.$$

FCH and MDH used 21 and 19 free parameters, respectively, which are identifiable, according to Shipley (2000).

Results

The distribution of *CopS* is showed in Fig 2. Most of the bucks (68.6%) had no copulations. The number of copulations per individual ranged from 0 to 43, and the distribution has high kurtosis (32.33) and skewness (4.99). The distribution of *CopS* is best fitted by a negative binomial distribution $(\chi^2 = 0.28,\ P = 0.595)$, which is much better supported than alternative models (ZINB, ΔAIC=33.39; ZIP, ΔAIC=152.47; Poisson, ΔAIC=535.02). Data transformation changes the discrete *CopS* distribution into a continuous one, which remains, however, non-normally distributed (Shapiro-Wilk Test: log(*CopS*+1), *W*=0.648, P<0.001; log(*CopS*+0.5), *W*=0.659, P<0.001; log(*CopS*+0.1), *W*=0.662, P<0.001; $CopS^{0.5}$, *W*=0.635, P<0.001).

Linear and Generalized Linear Models

The AIC and BIC values associated with LMs with untransformed response variables and GLMs are reported in Table 1. LM_1 and LM_{1r} have considerably higher AIC and BIC than GLMs. Among the different GLMs, $GLM_{2,r}$ exhibits the lowest AIC and BIC values, while the corresponding full model, $GLM_{5,r}$ has higher AIC and BIC values. $GLM_{4,r}$ presents the same AIC values as $GLM_{2,r}$, but a higher BIC values. As expected, MAMs show lower fit indexes than corresponding full models, except in Hurdle model. The different models identify different sets of significant variables, and the unstandardized coefficients for all models are given in S4 Table. In synthesis, among the eight variables considered, only *HS* and *CourtS (*except in GLM$_{5,r}$) are always detected as significant, whereas *TotS*, *Ds,* and LA_1 were only put into light by some of the GLMs. Note, however, that their estimates are nonsensical since they are always negative, whereas positive values are expected. This is an example of Simpson's paradox, which Pearl (e.g. Pearl 2016) has discussed as a common problem with non-SEM studies.

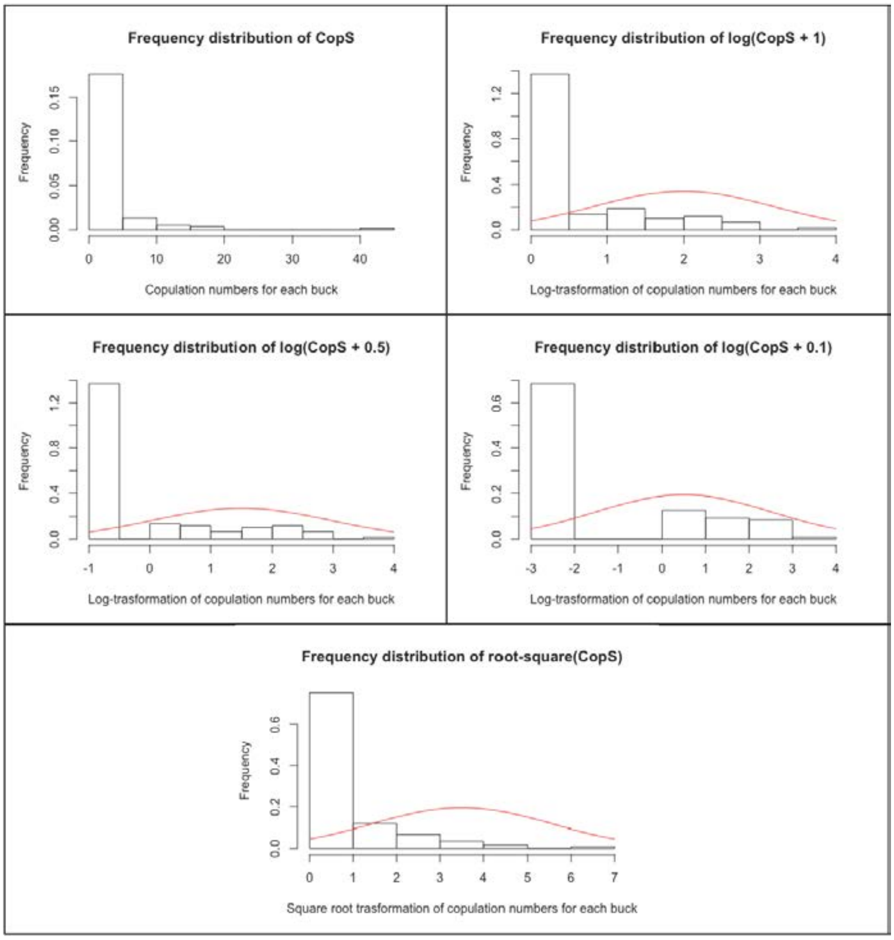


Fig 2. Frequency distribution of number of copulations achieved by each buck (*CopS*) before (upper left panel) and after transformation. The continuous red line shows the theoretical normal curve for reference.

Table 1. AIC and BIC values associated with linear (untransformed) and GLM models.

Model	Type	K	AIC	BIC
LM_1	*Normal*	9	662.6	687.5
$LM_{1,r}$	*Normal*	3	657.7	670.9
GLM_1	*Poisson*	9	283.2	308.2
$GLM_{1,r}$	*Poisson*	6	280.2	296.8
GLM_2	*Neg Binom*	10	224.7	252.4
$GLM_{2,r}$	*Neg Binom*	4	222.7	233.8
GLM_3	*ZIP*	10	285.2	313.0
$GLM_{3,r}$	*ZIP*	7	282.2	309.9
GLM_4	*ZINB*	11	226.7	257.2
$GLM_{4,r}$	*ZINB*	5	224.7	255.2
GLM_5	*Hurdle*	10	385.3	412.9
$GLM_{5,r}$	*Hurdle*	6	400.8	414.7

K = number of parameters in the model. Type indicates the distribution used.
The suffix r indicates reduced models

The models with linear transformed response variables (Table 2) have erratic AIC and BIC values varying from a minimum for $LM_{4,r}$ (AIC = - 67.6 and BIC = -59.3) to a maximum associated to $LM_{5.}$ (AIC = 347.7 and BIC = 372.6). AIC and BIC values vary in an unpredictable way depending on the value of the constant added to the transformed variable (or in the calculation of maximum likelihood in the case of the square root transformation). Due to the complete unreliability of data transformations, this approach will not be considered further in this paper.

Table 2. AIC and BIC values associated with linear models with transformed response variables.

Model	Transformation	K	AIC	BIC
LM_2	*log(x+1)*	9	250.2	275.1
$LM_{2,r}$	*log(x+1)*	3	249.4	257.7
LM_3	*log(x+0.5)*	9	157.5	182.4
$LM_{3,r}$	*log(x+0.5)*	3	156.7	165.0
LM_4	*log(x+0.1)*	9	-66.3	-41.3
$LM_{4,r}$	*log(x+0.1)*	3	-67.6	-59.3
LM_5	$x^{0.5}$	9	347.7	372.6
$LM_{5,r}$	$x^{0.5}$	3	344.7	353.0

K = number of parameters in the model. Transformation indicates the type of transformation applied to the dependent variable. The suffix *r* indicates reduced models.

Structural Equation Models

The variance-covariance/correlation matrix used in SEM and GSEM is reported in S5 Table.

To select the appropriate distribution of *CopS* for GSEM, we first selected the discrete distributions available both in Mplus and STATA. It resulted that only two of these distributions, Poisson and Negative binomial, were supported. According to the results of Table 1, we first tested the negative binomial distribution, but the model did not converge in either software. Thus we were forced to use the Poisson distribution.

If we implement the SEM for MDH with Mplus, convergence is not achieved, because the residual covariance matrix is not positive definite (Kolenikov & Bollen 2012) and the residual variances associated with LA_1 have negative values. Note that the AIC values yielded by Mplus are biased. Indeed, in GSEM the convergence of the MDH model is only achieved by fixing the path-coefficients for *Dom*, $LA_{1,}$ and *HS* to a predefined value. The MDH model (SEM or GSEM) does not converge with STATA. With these problems of convergence, GSEM, was always better than SEM (ΔAIC = 433.4 and ΔBIC = 436.2). On the contrary, the FCH converges using both SEM and GSEM. Even for FCH, GSEM provided a better fit than SEM (ΔAIC = 438.9, ΔBIC = 441.7). In synthesis, this analysis shows that FCH is always preferred to MDH by having lower AIC and BIC values both when fitted using SEM and GSEM (ΔAIC and ΔBIC >140 always). Due to these results, the MDH model will not be considered in the following analyses. Path coefficients for GSEM-FCH models are shown in Table 3. All coefficients are highly significant ($P<0.003$). Noteworthy, the path coefficient for ASS_T is positive and not negative as expected.

Model comparisons

The comparison of the models is reported in Table 4. It clearly appears that the precision of MAM models for LMs and GLMs is higher than that of the corresponding full models. Considering the median CV values, the two less precise models are GLM_4 (median CV = 1.089) and LM_1 (median CV=0.828), while the more precise models are $GLM_{4,r}$ (median CV=0.162) and $GLM_{2,r}$ (median CV=0.148). LMs and GLMs were clearly outperformed by both the SEM (median CV=0.079) and, to a larger extent, by GSEM (median CV = 0.059), whose coefficient CV values range from 0.02 to 0.319.

Table 3. Standardized path coefficients, SE, and p-value for FCH in GSEM.

Variables	**Path coefficients**	**GSEM**	
		Estimate ± SE	**P**
Mating success (η_2)			
HS	λ_5	0.630 ± 0.065	<0.001
CourtS	λ_6	0.896 ± 0.019	<0.001
CopS	λ_7	2.387 ± 0.138	<0.001
Lek attendance (η_1)			
LA_1	λ_3	0.936 ± 0.035	<0.001
LA_2	λ_4	0.969 ± 0.038	<0.001
Antler shape (ξ_1)			
ASS_T	λ_1	0.480± 0.038	<0.001
TotS	λ_2	0.379 ± 0.121	0.002
η_1 on ξ_1	γ_1	0.585 ± 0.194	0.003
η_2 on η_1	β_1	0.330 ± 0.093	<0.001

Variables and symbols are detailed in the text.

Table 4. Summary results of LM, GLM, SEM, and GSEM.

Model	***K_1***	***ASS_T***	***TotS***	***Dom***	***Ds***	***LA_1***	***LA_2***	***HS***	***CourtS***	***Median***
LM_1	2	1.883	1.007	0.645	0.859	0.798	1.600	0.182	0.339	0.828
$LM_{1,r}$	2							0.171	0.286	0.228
GLM_1	5	0.624	0.227	8719	0.365	0.369	1.177	0.098	0.103	0.367
$GLM_{1,r}$	5		0.235		0.262	0.214		0.086	0.097	0.214
GLM_2	2	4.569	0.783	3.369	1.299	0.613	2.033	0.199	0.132	1.041
$GLM_{2,r}$	2							0.178	0.119	0.148
GLM_3	5	0.625	0.227	2311	0.335	0.368	1.176	0.098	0.103	0.351
$GLM_{3,r}$	5		0.235		0.262	0.214		0.086	0.097	0.214
GLM_4	2	4.946	0.838	3.395	1.341	0.644	2.204	0.219	0.144	1.089
$GLM_{4,r}$	2							0.193	0.132	0.162
GLM_5	3	0.508	0.296	0.826	2.75	0.605	100	0.128	0.886	0.715
$GLM_{5,r}$	3	0.456	0.520					0.097		0.456
SEM-FCH	7	0.079	0.312			0.051	0.046	0.143	0.131	0.079
GSEM-FCH	7	0.079	0.319			0.037	0.039	0.103	0.021	0.059

On the left: type of model, number of significant ($P<0.05$) coefficients. On the right: coefficient of variation (CV) of regression parameters and their median. MAMs are denoted by the suffix r. Variable names are detailed in the text. All models have the same numbers of observations (N=118). K1 is the number of significant regression coefficients.

Comparable results are obtained when analysing the distribution of residuals (Table 5, Fig 3). In LMs, the variance is very large, and the distribution is strongly leptokurtic with heavy tails (Fig 3). As a comparison, statistics of the distribution of residuals for LMs with transformed response variables are shown in S6 Table.

These distributions are characterised by large variances and kurtosis, and none is centred on zero.

Table 5. Mean, variance, and kurtosis for residual distributions of the different models considered in this paper.

Model	***Mean***	***Var***	***Kurtosis***
LM_1	0	13.92	29.96
$LM_{1,r}$	0	14.78	30.58
GLM_1	-0.26	1.18	9.23
$GLM_{1,r}$	-0.27	1.2	9.32
GLM_2	-0.21	0.32	9.45
$GLM_{2,r}$	-0.23	0.33	8.30
GLM_3	-0.10	1.32	9.86
$GLM_{3,r}$	-0.11	1.30	9.44
GLM_4	-0.08	0.36	14.44
$GLM_{4,r}$	-0.09	0.38	18.41
GLM_5	0.01	1.28	7.55
$GLM_{5,r}$	0.01	1.37	7.22
SEM – FCH	0	6.10	32.56
GSEM – FCH	0.14	0.28	6.84

Models are in Table 1.

GLMs perform better than LMs (Table 5), distributions remain leptokurtic, but variances are smaller, and the mean is slightly biased low (Fig 3a and 3b). The residuals associated with SEM-FCH (actually to the relationship between *CopS* and η_2), although their mean is close to 0, have a strongly leptokurtic distribution and have a variance much larger than that of GLMs (but not LMs). Finally, the residuals associated with GSEM-FCH have a low variance and the least value of kurtosis among the studied models.

Interestingly the number of regression coefficients that are significant is maximal in SEM and GSEM (Table 4). Since results indicate that GSEM-FCH is the model more appropriate for our data (lower AIC/BIC, lower residuals' variance, and lower CV median), it is interesting to investigate total effects (cfr. S3 Text) for this model (Table 6). Noteworthy, the impact of ξ_1 and η_1 on *CopS* is of similar size with respect to η_2, while ξ_1 and η_1 have much smaller effects on *CourtS* or *HS* than η_2, which suggests a remote causation for *CopS*. The impact of ξ_1 on both ASS_T and *TotS*, but to different degree, is more relevant for ASS_T than *TotS*.

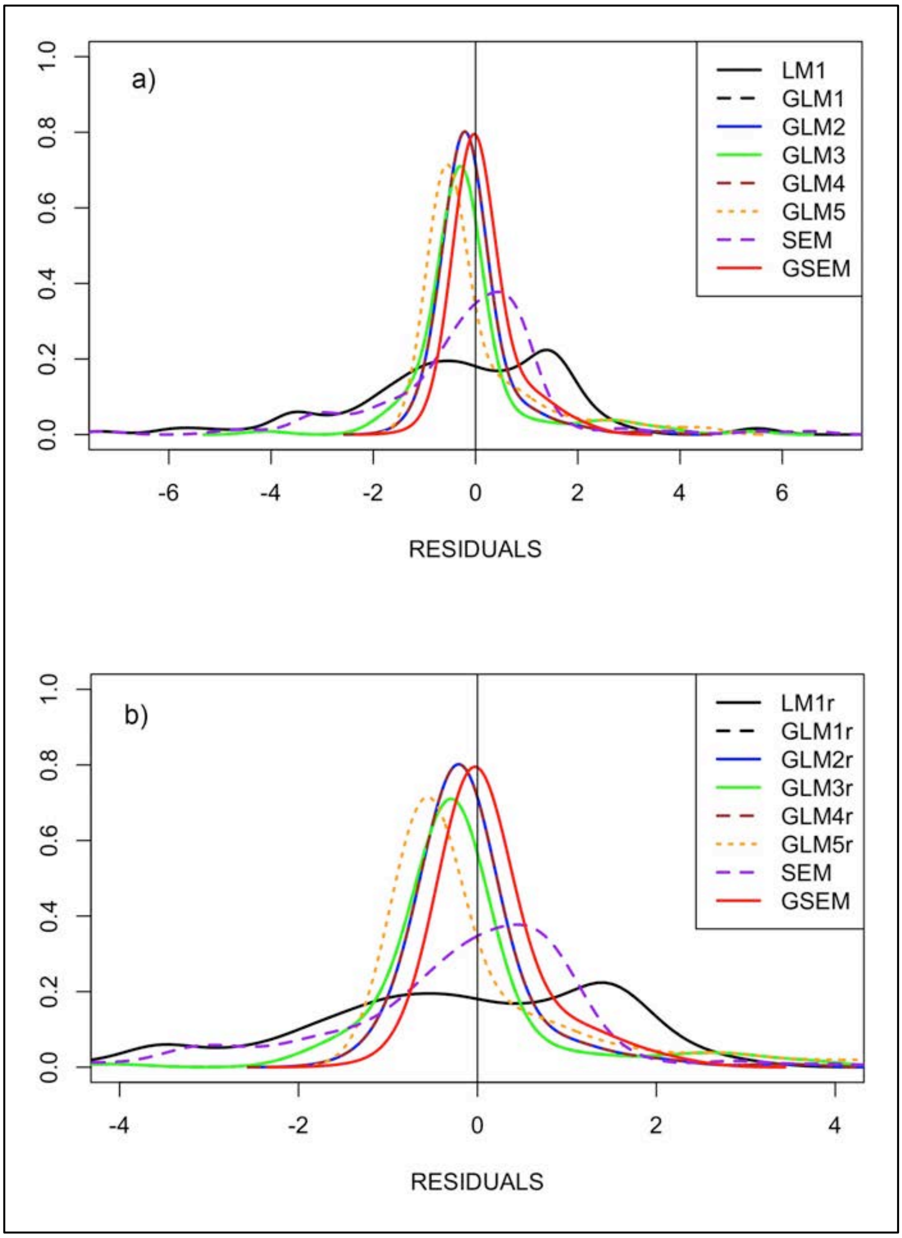


Fig 3. Model validation graph. a) Distribution of standardized residuals of GLMs, SEM, and GSEM models. For LMs and GLMs, both full (a) and reduced models (b) are shown. Models are in Table 1. The respective descriptive statistics of the different distribution models considered in this paper are reported in Table 5.

Table 6. Total effects of GSEM in FCH model.

Manifest Variables	**GSEM**		
	ξ_1	η_1	η_2
ASS_T	0.480		
TotS	0.379		
LA_1	0.548	0.936	
LA_2	0.567	0.969	
HS	0.122	0.208	0.630
CourtS	0.173	0.296	0.896
CopS	0.193	0.330	2.387
Latent Variables			
ξ_1		0.585	0.193
η_1			0.330

Discussion

The data collected at Castelporziano on the mating behaviour of fallow bucks represents a typical example of the many studies performed on the leks of this species (Clutton-Brock et al. 1988; Fiske et al. 1998; Focardi & Tinelli 1996b; Apollonio et al. 2014) and other species of vertebrates (Sardell et al. 2014; Fiske et al. 1998; Kokko et al. 1998). These behavioural studies are important not only to identify the proximate causes of mate selection, but also for determining the intensity of sexual selection and understanding the evolution of exaggerated traits in males.

A literature review (cfr. S2 Text and S2 Table) allowed us to select the more popular methods used in previous research and to contrast them with innovative GSEMs. The use of the same dataset to compare different statistical methodologies is useful for evaluating their relative efficiency in data fitting. In general, LMs appear to be severely biased, and although GLMs may improve the reliability of the results, they overlook several important effects and the estimated coefficients still have low precision, which severely jeopardizes their predictive capacity. It is worth stressing that data transformation is not appropriate to normalize data distribution, since results appear extremely sensitive to the specific function used. This problem is exacerbated by the large number of zeros in the distribution of male copulatory success.

The introduction of GSEMs in the analysis of lek mating appears to represent a relevant leap ahead in the field. Our study provided evidence of several advantages of GSEMs compared to GLMs. First, the collinearity of predictors is no longer a nuisance provided that an appropriate measurement model is built, so we save part of the information collected in the field, which is usually lost in GLMs to reduce variance inflation (Zuur et al. 2010). Second, GSEMs are a flexible tool since they allow contrasting different casual models (e.g. using AIC, BIC, or other fit indexes) which must be formulated a–priori. In comparison to both LM and GLM, a proactive model formulation improves the awareness of the biological significance of the mechanism to be tested and allows scholars to modify a basic theoretical construct by introducing specific paths which are known or thought to be relevant in each particular study condition.

This feature of SEMs allows us to include both general theoretical statements and specific conditions in the same model, which are then evaluated together. The publication of the variance-covariance matrix has the advantage of allowing other scholars to replicate the results easily and to propose different theoretical models pertinent to the system of interest, and in doing so, improve the transparency of the research and the full reproducibility of the results. However the availability of rough data can be useful to adjust the standard errors. Finally, SEM/GSEM help to control for measurement errors, a much neglected flaw in most quantitative analyses.

GSEM represents a bridge between the descriptive approach developed in LM and GLM and experimental tests with manipulative treatments; indeed the consistency of alternative causal paths can be tested, and when possible, the results can be used to develop more stringent experiments.

The importance of using GSEMs is well represented by the between-method comparisons reported in this study. First, we were able to show that, with respect to GLMs and even more to LMs, GSEMs suggest the potential influence of a larger number of predictors, in other words more informative models can be developed. This may have a strong impact on the interpretation of the study. For instance, both LMs and GLMs (except for the Poisson models) were unable to detect any effect of predictors referring to male dominance, which are however present, albeit with a small effect. Indeed in the literature, several authors were unable to detect these effects at all (e.g. Rintamaki et al. 2001; Kervinen et al. 2012; Apollonio et al. 1989; Loyau et al. 2007).

The second relevant aspect of GSEMs is the increased precision of the estimates of the regression coefficients. For several predictors GLMs yielded CV values >50% which are clearly unacceptable, while with GSEMs, CVs were often <10%, a precision we consider “acceptable” for a field study. The analysis of residuals in GSEMs and GLMs confirmed that the former allowed a better fitting of the data than the latter.

While these results are not meant to disprove the available results about lek breeding of fallow deer based on linear models, the analysis of our dataset illustrates some advantages in using GSEMs for discrete responses. SEMs are more flexible and have more parameters than GLMs and may better fit the data of interest. Indeed, the formal definition of contrasting working hypotheses, such as FCH and MDH in this study, is illustrative of the potentiality of SEM for hypotheses testing. On the other hand, with respect to LMs and GLMs, SEM are data hungry and Shipley (2000) gives a rule of thumb to decide the number of parameters that can be safely estimated given a certain sample size.

The practical use of GSEM presents several difficulties. The main problem is that the likelihood of SEMs with latent variables is generally multimodal, and there is a need for a general algorithm to locate the global maximum. Moreover, the algorithm sometimes does not converge to a proper solution and this usually suggests that the model is not identifiable (at least in some parts). A partial remedy is to include reasonable identifiability constraints. In path analysis or with GLMs, the problems of non-convergence are generally absent.

One drawback that may limit a wider diffusion of GSEM is that the possibility of modelling non-normal variables is not yet implemented in widespread statistical packages, such as SAS, R, or S-plus. In this paper, GSEMs have been implemented in Mplus and STATA. We support the importance of using both packages, because they present complementary advantages and disadvantages. For instance STATA provides case-specific residuals, which are not outputted by Mplus, but Mplus returns the standardized path coefficients and total, direct, and indirect effects which STATA does not compute. The use of Mplus requires caution, because to get convergence, it automatically constrains the value of some path coefficients to be one. In STATA, constraints have to be specifically applied, which is a feature that improves awareness for the user. In our experience, STATA is much slower than Mplus, but it is well-documented; in some cases STATA, unlike Mplus, failed to converge (e.g. with MDH). However, STATA implements only a limited GSEM procedure, for example it does not support ZIP or ZINB distributions despite the greater flexibility in model specification.

The analyses in this paper were developed under a frequentist approach. A Bayesian analysis of our data with GSEM is outside the scope of the present study and would require further research especially as far as the choice of priors is concerned. For an introduction to Bayesian SEMs see Kaplan & Depaoli (2012).

The importance of this study lies in the fact that, to our knowledge, it is the first comparative study of SEM and GSEM models. We believe that past work should be reviewed in the light of the results obtained here. Specifically, the results from studies using LMs should be considered with great caution, particularly in those cases where assumptions were clearly violated and transformations to normalise non-normal variables were applied. Interestingly, Grace et al. (2016) analysed the species richness-productivity relationships using SEM and showed that an integrative model has an higher explanatory power than traditional linear models, since SEM allows us to integrate competing hypothesis into a single model. Furthermore, SEMs help to solve the Simpson's paradox (Pearl et al. 2016). Finally, it is important to stress that the use of GSEMs can be extended to other behavioural and ecological contexts characterised by non-normal distributions of variables. SEMs are getting traction in behavioural studies and in ecology. According to the WOS (accessed on the 13/5/2016), the number of ecological and zoological papers using SEM is increasing by 7% per year. Thus, GSEM can find wider and wider opportunities for application. In particular, the possibility of using SEMs to test hypotheses in competition and investigate both remote and proximate effects is of particular interest in ecological and evolutionary studies. The present study can therefore stimulate the application of GSEM to different study cases.

Data accessibility

The Castelporziano data set used in this paper are available in https://doi.org/10.1371/journal.pone.0181305.s001.

The Mplus and STATA code used to generate the models are available in online S4 Text. The R code used to generate part of the results are available as part of online S5 Text.

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Supporting Information

S1 Text

Details on data validation and measures computation

Adult bucks were individually identified using a database of antler morphology. Fallow deer were videotaped from a high seat at the margin of the lek area, and pictures of antlers were used to estimate the number of small (*SS*) and large (*LS*) spellers, according to Pelabon & Joly (2000). All antlers missing tines, without palms, or were broken were excluded from the analysis. Two variables were used: a) the total number of spellers (*TotS*) and b) a measure of fluctuating asymmetry for small spellers (Møller 1990; Møller *et al.* 1996), computed as:

$ASS_T = |SS_r - SS_l|$,

where SS_r and SS_l are the number of small spellers on the right and the left antler, respectively.

Animals were systematically observed from dawn to dusk (38 days, 396 hours in 1991, 40 days, 337 hours in 1992) at hourly intervals (the data were collected at discrete intervals), and the position of each identified buck in the lek, its activity, and the number of females and fawns within its territory were recorded. All fights and copulations were also noted. A total of 695 fights were observed in 1991 and 186 in 1992. Only the fights which ended with the victory of one of the rivals were considered.

To validate the estimates of the number of large (*LS*) and small (*SS*) spellers, we used a set of 69 cast antlers, collected at Castelporziano in the period 1991-1998, for which pictures drawn while animals were in the lek were also available. One operator evaluated the number of large and small spellers from drawings, while another independently obtained the same information from photos of the casted antlers. The results showed that the two estimates of the number of spellers were consistent (Pearson's correlation; *SS*: r=0.57, N=23, P=0.004; *LS,* r=0.67*,* N=23*,* P=0.005). When casted antlers were found, a complete set of biometric measurements were also taken, including their weight (*AW*).

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S1 Table.

Complete list of variables and their definitions used in the models GLMs, SEMs and GSEM.

Model's variables name	***Description of variables***
ξ_1	Latent variable "Antler shape" is positively related to both number of spellers (*TotS)* and their fluctuating asymmetry (ASS_T).
ASS_T	The fluctuating asymmetry of small antler's spellers.
TotS	The total number of small and large antler's spellers.
ξ_{1a}	Latent variable "Dominance rank" is positively correlated to dominance indexes (*Ds, Dom*).
Ds	The David's score, *Ds,* (*Gammel et al. 2003*) divided for the total number of bucks of each year.
Dom	Dominance Index Clutton-Brock et al. (*1979*) divided for the total number of bucks of each year.
η_1	Latent variable "Lek attendance" is correlated to lek attendance index (LA_1, LA_2).
LA_1	The number of days in which the animal was present in the lek.
LA_2	The total number of days of presence/territory in different locations of the same lek.
η_2	Latent variable "Mating success" is related to harem size, courtship behaviour and buck's copulatory success (*HS, CourtS, CopS*).
HS	The mean number of females in a male's territory.
CourtS	The fraction of courtship events terminated with a copulation (number of copulations /number of courtship events, for every male).
CopS	The total copulatory success of the *i*-th buck in one rut.

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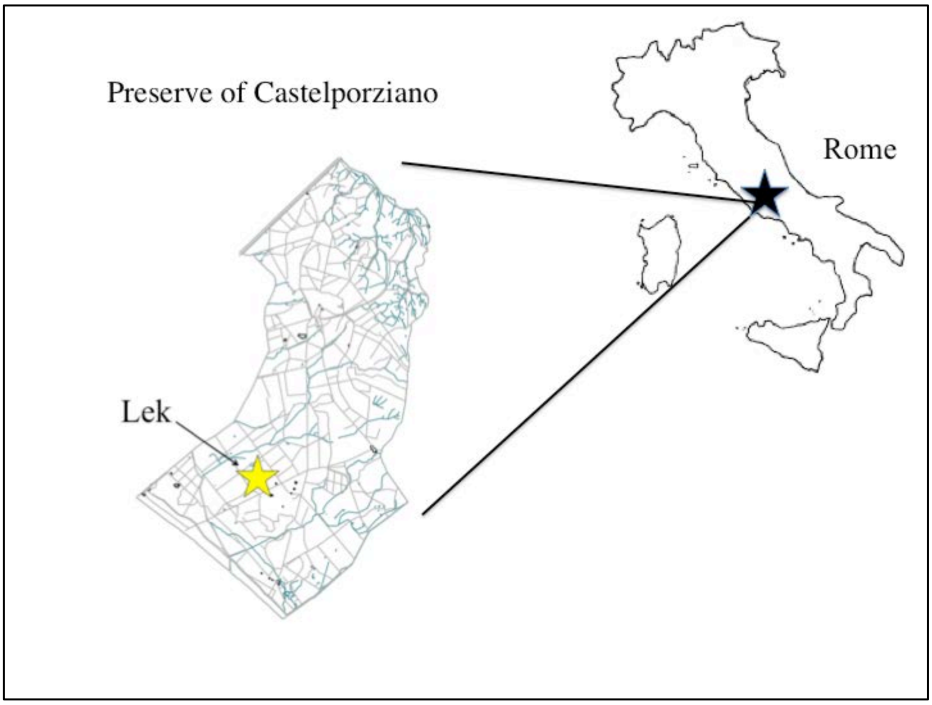
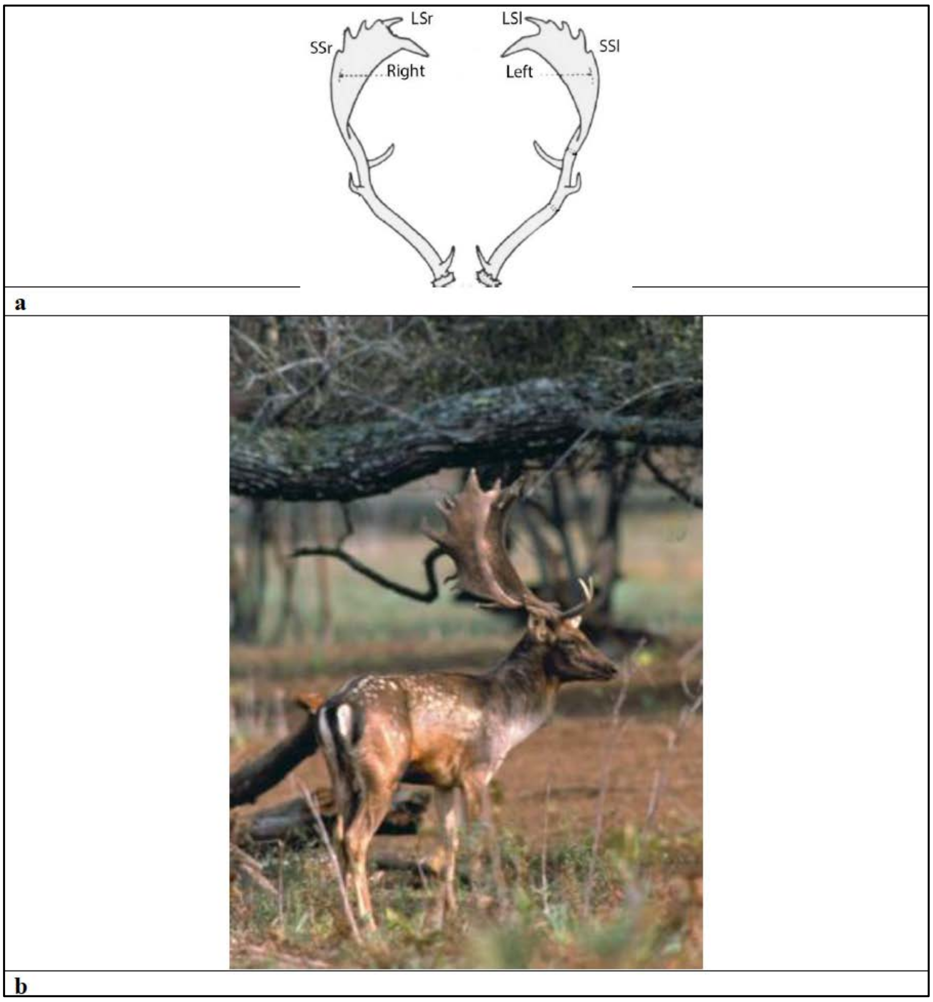
S1 Fig

Study area in Castelporziano (Rome, Italy)



S2 Fig
Phenological characters of fallow deer buck (*Dama dama*) in Castelporziano (Rome, Italy)



S1 Figure 2. *a) Let us define* SS_r *and* LS_r *the small and large spellers of the right antler, respectively and* SS_l *and* LS_l *those of the left antler and consequently* RS_T *like the right total spellers and* $LeftS_T$ *the left total spellers. We may compute global measures for antlers size:*
$SS_T = SS_r + SS_l$, $LS_T = LS_r + LS_l$, $LeftS_T = LS_l + SS_l$, $RS_T = LS_r + SS_r$, $TotS = RS_T + LeftS_T$ *for total spellers (small and large).b) Male fallow deer in Castelporziano.*

S2 Text

Literature review

We reviewed the main statistical methods used, pinpointing their weaknesses and the solutions adopted. The choice of paper included in the meta-analysis was carried out by searching on the Web of Science^{TM} in on February 2016 for the 1980-2016 period, using as keywords: lek (AND deer OR antelope OR mammal) AND (mating success OR copulatory success). The search found 109 papers among which we selected those whose working hypotheses were comparable with those of our paper. We analyzed a total of 31 papers, listed in S2 Table.

In the majority of cases (n=15), some type of multiple regression was used. There were three cases where authors only presented descriptive statistical analyses, in three other cases chi-square or Fisher's tests were used, three papers used simulations and ten other studies employed Pearson's or Spearman's rank correlations, without any attempt to control for spurious correlations.

In eleven of the papers that presented a linear (LM) or a linear mixed-effects model (LMM), the response variable (the number of copulations achieved by each male) was used as is (1) or log/square-root transformed. One author used a logistic regression model, after transforming copulation numbers into a binary variable (present/absent). Five papers employed GLM (or GLMM) explicitly using non-normal distributions (Poisson or Zero Inflated Poisson, ZIP) for the response variable. In one case the variable was normalized using Blom's score. Finally, only one paper used path analysis with manifest variables, but with a violation of normality assumption of the response variable. In this case, the authors used a transformation of the original data based on Blom's scores, a rank procedure that yields normally-distributed variables.

S2 Table.
List of the main papers on copulatory success in lek mating (from 1980 to 2016), with a synthetic description of methods used.

Paper	***Statistical Method***	***Description***
Clutton-Brock (1988)	a) Correlation b) Non-parametric test	Dependent variables were tested for normality

Paper	***Statistical Method***	***Description***
Apollonio et al. (1989)	LM	Square root transformation of response variable (copulatory success).
Goslin & Petrie (1990)	Chi-square test	
Thirgood (1990)	a) Descriptive Statistical analysis. b) Chi-square test	Dependent variables were tested for normality
Balmford et al. (1992)	Spearman's correlation	The distribution of male reproductive success fitted to a Poisson.
Deutsch & Nefdt (1992)	LM	Log transformation of copulatory success log(copulations+1)
Byers et al. (1994)	Pearson's correlation	Normalized copulatory success using mean number of copulation per rut attained per male aged 1-9 years.
Deutsch (1994)	a) Descriptive Statistical analysis b) Pearson's correlation	
Marks et al. (1994)	a) Simulations b) LM	Standardized male mating success.
Focardi & Tinelli (1996a)	a) Simulations b) LM c) Test for Poissonian process.	1) CV for standardized copulatory success. 2) The distribution of male copulatory success fitted as a Poisson.
Mackenzie et al. (1995)	Theoretical model	Random female mating assumed to be Poisson distributed.
Focardi & Tinelli (1996b)	Path analysis with manifest variables	Rank procedure for non-normal variable transformation.
Hirth (1997)	a) Descriptive statistical b) Test of density hypothesis	
Kokko et al. (1998)	LM	Log-trasformation of copulatory success.
McElligott et al. (1998)	a) Non parametric-test b) Sperman's rank correlation	Rank correlation.
Fiske et al. (1998)	a) Sperman's rank correlation b) Power of test	Rank correlation.
Kokko et al. (1999)		Memory of the lekking system as a determinant of the male mating success Power test
McElligott et al. (1999)	LM	
Isvaran & Jhala (2000)	Descriptive statistical	
McElligott et al. (2001)	a) Kendall rank-order correlation coefficient b) Partial rank-order correlation coefficient	Pearson's correlation to investigate collinearity

Paper	***Statistical Method***	***Description***
Rintamaki et al. (2001)	a) GLM b) Logistic regression	1) Poisson distribution for response variable, and logarithm as the link function. 2) Response variable transformation from copulation number to a binomial variate (0 or 1).
Bro-Jørgensen (2003b)	Non-parametric test	1) Friedman tests to avoid pseudoreplication. 2) Dunnett's tests for multiple comparisons
Loyau et al. (2007)	GLM	Poisson distribution of response variable and logarithm as the link function.
Fricova et al. (2008)	GLMM (Generalized Linear Mixed Models)	Count distribution of response variable and locations as random factor.
Bro-Jørgensen (2008)	Logistic Regression Model.	Response variable transformation from copulation number to a binomial variate (0 or 1).
Bro-Jørgensen et al. (2011)	REML (Linear Mixed Model)	1) Model with male ID as random factor 2) Covariates (age) included as quadratic expression in order to allow for the non linear relationship with mating success.
Ciuti et al. (2011)	LME (Linear Mixed Effects Model)	1) Model with male ID as random factor 2) Log-trasformation (copulations + 1)
Ciuti et al. (2011b)	LME (Linear Mixed Effects Model)	1) Data log-transformation of dependent variables 2) Dependent variables were successful tested for normality and homoscedasticity after trasformation.
Kervinen et al. (2012)	ZIP (Zero Inflated-Model)	Model with Poisson error distribution.
Dakin & Montgomerie (2013)	GLMM (Generalized Linear Mixed Models)	1) Fourth root trasformed standardized copulation number. 2) Use of ZIP and male ID as random factor.
Sardell et al (2014)	a) Correlation b) LM c) Chi-square	

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Byers JA, Moodie JD & Hall N. (1994). Pronghorn females choose vigorous mates. *Animal Behaviour*, 47, 33-43.

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S3 Text

Structural Equations Model (SEM) and Generalized Structural Equation Model (GSEM)

A detailed presentation of structural equations may be found in Shipley (2000). The development of a structural model requires the *a-priori* definition of links among model variables in the form of a regression equations system. The goal of this class of models is minimize the difference between estimates and expectations variance-covariance matrix of data.

We assume causal relationships going from the latent variable to the manifest variables in its block. Latent variables are unobserved factors denoted, $\eta_1, \eta_2, \ldots, \eta_n$, that represent an hypothetical construct that can be inferred by the way it influences manifest or observed variables (continuous, $Y_i = y_1, y_2, .., y_n$) (Shipley 2000; Pugesec 2003; Muthén & Asparouhov 2015).

A SEM model is composed by two sub-models: a measurement model that describes the relationships between latent variables and their manifest variables and a structural or causal model that constitutes a directional chain system that describes the relationship between the constructs of theoretical interest (latent variables) using *path diagrams* (S3 *Fig*).

Structural coefficients (γ, β) represent the effects of each independent variable on the dependent variable.

A manifest variable, in a SEM with latent variables, plays a role of endogenous variable if it is predicted by another variable in the model and is therefore a response variable; it is assumed to be generated as a linear function of its latent dimension and the residual error term represents the imprecision in the measurement process. An exogenous variable whose variation is not explained in a model (i.e. antler shape and FA of small spellers).

In synthesis (Bollen, 1989; Chen *et al.* 2001; Kolenikow & Bollen, 2012) a SEM of a LISREL type is composed by a system of three equations given by:

$$\eta = a_{\eta} + \boldsymbol{B}\eta + \boldsymbol{\Gamma}\xi + \zeta,$$

$$x = a_{x} + \boldsymbol{\Lambda}_{x}\xi + \delta, \qquad (1S3\ eqns)$$

$$y = a_{y} + \boldsymbol{\Lambda}_{y}\eta + \varepsilon .$$

The first equation represents the causal model, x and y represent the measurement model. The standard assumptions are made that ξ, ζ, δ, ε are uncorrelated, and have covariance matrices Φ, y_{ζ}, y_{δ}, y_{ε}. Based on the SEM model the implied covariance matrix of $p = (x', y')'$ is given by $\sum(\theta)$where the parameter vector $j$contains the intercept vectors a_{x}, a_{y}, The $\boldsymbol{B}$ and $\boldsymbol{\Gamma}$ matrices, are the regression parameters required to be estimated. The $\boldsymbol{\Lambda}_{x}$ and $\boldsymbol{\Lambda}_{y}$ are the factor loadings for η and ξ. The ε and δ vectors are the measurement errors for y and x respectively and ζ is referred to the disturbance.

The researcher is interested in parameter estimates of the coefficients and the overall goodness of fit test of the model:

$$H_0 : COV[p] = \sum(\theta)$$

$$H_1 : \forall\theta\ COV[p] \neq \sum(\theta)$$

with dimension $.\pi *= \pi(\pi + 1)/2.$

For instance, in the following equation:

$$y_4 = \lambda_1 y_1 + \lambda_2 y_2 + \ldots\ldots + \varepsilon_{y4},$$

y_4 represents the dependent variable,y_1, y_2,.., etc. the independent variables and ε_{y4} the error term for y_4; λ_1, λ_2 are the parameters to be estimated. Given a set of dependent variables y_1, y_2, ecc… our aim is to minimise the difference between experimental and predicted variance-covariance matrices $(COV\,[p] = \sum(\theta))$

The fundamental hypothesis underlying these approaches is that the implied covariance matrix of the manifest variables is a function of the model parameters.

SEM are related to factorial analysis where underlying and unknown factors are evidenced by the correlations among the manifest variables. On the other side a modification in the structural part implies a difference about the theoretical hypothesis to be tested.

In a structural equation, standardized parameters λ_1, λ_2.., represent the effects of each independent variable on the dependent variable. Beside such direct effects we can also compute indirect effects. Consider the system of structural equations:

$$y_3 = \lambda_1 y_1 + \lambda_2 y_2 + \varepsilon_{y3},$$

$$y_4 = \lambda_3 y_3 + \varepsilon_{y4}.$$

It is clear that y_3 has a direct effect on y_4 but y_1 may also indirectly influence y_4 via y_3 with intensity $\lambda_1\lambda_3$. Our aim is to evaluate total effects (direct and indirect) of manifest variables on copulatory success calculated by multiplication structural coefficients (total effect= direct effect + indirect effect).

SEM can test different working hypothesis, by comparing alternative models. A straightforward method to evaluate model fitting is to inspect the distribution of standardised residuals. Parameter estimation is performed by maximum likelihood (ML) estimation or maximum likelihood robust (MLR) estimation. The unknown parameters of the model are estimated so as to make the variances and covariances that are reproduced from the model in some sense close to the observed data. A good model would allow very close approximation to the data. The literature is full of discussion about the opportunity of using goodness-of-fit indexes (see Shipley 2000:188-194).

Since we compared non-nested models with identical number of variables and sample size, we adopted the AIC (Akaike, 1974).

SEM with Generalized response (GSEM) use a generalized measurement part with ordered categorical and continuous variables, grouped together also in the same latent construct, to emphasize the nature of the Poissonian process (ZIP) or Negative Binomial process (ZINB), that produce count variables like the number of success, the rate of courtship, the number of female and finally copulatory success (Muthén & Muthén, 2015, Mplus, 7.4 release*)*.

For instance, a model with two latent variables, two exogenous and two endogenous manifest variables described by the following system of equations:

$$
\begin{aligned}
\eta_1 &= \gamma \xi_1 + \zeta_1 \\
x_1 &= \lambda_1 \xi_1 + \delta_1 \\
x_2 &= \lambda_2 \xi_1 + \delta_2 \\
y_1 &= \lambda_3 \eta_1 + \varepsilon_1 \\
y_2 &= \lambda_4 \eta_1 + \varepsilon_2
\end{aligned}
\qquad (2S3\ eqns)
$$

can be graphically represented by the path diagram in S3 Fig. Variable x_1 and x_2 are manifest exogenous variables because they are not explained by the model, while y_1 and y_2 depend on the structure of the model and are called manifest endogenous variables. We denote by ξ_1 the latent exogenous variables, and by η_1 the latent endogenous variable. Variables δ_1, δ_2, ε_1, ε_2 and ζ_1 are typically assumed normally-distributed error terms while γ, λ_1, λ_2, λ_3 and λ_4 are the path coefficients to be estimated. The variance of latent variables was set equal to 1.

References

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Chen F, Bollen KA, Paxton P, Curran P, & Kirby J. (2001) Improper solutions in structural equation models: Causes, consequences, and strategies. *Sociological Methods and Research*, 29, 468-508.

Kolenikov S & Bollen KA. (2012) Testing negative error variances: Is a Heywood case a symptom of misspecification? *Sociological Methods and Research*, 41, 124-167.

Pugesek BH, Tomer A, Eye A. (2003) Structural equation modeling. Cambridge University Press, Cambridge.

Muthén B & Asparouhov T. (2015) A general structural equation model with dichotomus, ordered categorical, and continuous latent variable indicators. *Psychometrika,* 49, 115-132.

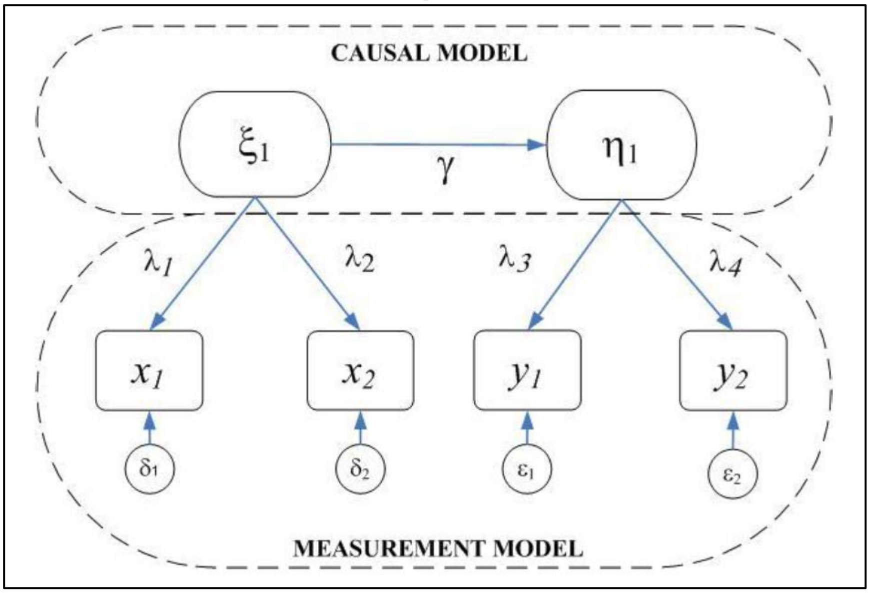
Muthén B & Muthén L. (2015) Mplus 7 ver. 7.4 Copyright © 2012-2015.

Shipley B. (2000) Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference. Cambridge University Press.

S3 Table
Table with the complete list of variables name of the models and the respective path coefficients.

Model's variables name	***Path Coefficients***
ξ_1	
ASS_T	λ_1
TotS	λ_2
ξ_{1a}	
Ds	λ_{1a}
Dom	λ_{2a}
η_1	
LA_1	λ_3
LA_2	λ_4
η_2	
HS	λ_5
CourtS	λ_6
CopS	λ_7
Structural or Causal Model	***Path Coefficients***
Female Choice Hypothesis	
ξ_1 –> η_1	λ_1
η_1 –> η_2	β_2
Male Dominance Hypothesis	
ξ_{1a} –> η_1	λ_{1a}
η_1 –> η_2	β_1

S3 Fig
An example of SEM



S3 Figure. *The SEM model of 2S3 eqn. The causal model is formed by two latent variables (ξ_1 exogenous and η_1, endogenous), while γ represents the causal link according to the arrow's direction. Measurement model: x_1, x_2 (exogenous) and y_1, y_2 (endogenous) are manifest variables, δ_1, δ_2, ε_1 and ε_2 are their error terms, λ_1, λ_2, λ_3 and λ_4 are the factors loading.*

S4 Text
Mplus and STATA codes used to generate SEM and GSEM.

STATA CODE
SEM INSTRUCTIONS FOR FEMALE CHOICE MODEL

```
clear
use "/Users/Desktop/GSEM/datiSEM.dta"
set more off

sem (xi@1 -> asst, ) (xi -> tots, ) (xi -> eta1, ) (eta1 -> la1, )
(eta1 -> la2, ) (eta1 -> eta2, ) (eta2 -> hst, ) (eta2 -> cops, )
(eta2 -> courts, ), vce(robust) latent(xi eta1 eta2 ) cov( xi@1
e.eta1@1 e.eta2@1) nocapslatent
predict ch hh oh, xb(cops hst courts)
predict e1h e2h, xblatent(eta1 eta2)
gen res =  cops - ch
summarize res
```

SEM INSTRUCTIONS FOR DOMINANCE MALE MODEL

```
use "/Users/Desktop/GSEM/datiSEM.dta"
sem (xi@1 -> dom, ) (xi -> ds, ) (xi -> eta1, ) (eta1@1 -> la1, )
(eta1 -> la2, ) (eta1 -> eta2, ) (eta2@1-> hst, ) (eta2 -> cops, )
(eta2 -> courts, ), vce(robust) difficult latent(xi eta1 eta2 ) cov(
xi@1 e.eta1@1 e.eta2@1) nocapslatent
```

GSEM INSTRUCTIONS FOR FEMALE CHOICE MODEL

```
clear
use "/Users/Desktop/GSEM/datiSEM.dta"
set more off
gsem (xi@1 -> asst, ) (xi -> tots, ) (xi -> eta1, ) (eta1 -> la1, )
(eta1 -> la2, ) (eta1 -> eta2, ) (eta2 -> hst, ) (eta2 -> cops,
family(poisson) link(log)) (eta2 -> courts, ), difficult latent(xi eta1
eta2 ) cov( xi@1 e.eta1@1 e.eta2@1) nocapslatent
predict coppa, mu outcome(cops)
predict latta, latent(eta2)
gen rr =  cops - coppa
summarize rr
```

GSEM INSTRUCTIONS FOR DOMINANCE MALE MODEL

```
use "/Users/Desktop/GSEM/datiSEM.dta"
gsem (xi@1 -> dom, ) (xi -> ds, ) (xi -> eta1, ) (eta1@1 -> la1, )
(eta1 -> la2, ) (eta1 -> eta2, ) (eta2@1-> hst, ) (eta2 -> cops,
family(poisson) link(log)) (eta2 -> courts, ), vce(robust)
intmethod(ghermite) difficult latent(xi eta1 eta2 ) cov( xi@1
e.eta1@1 e.eta2@1) nocapslatent
predict copsa, mu outcome(cops)
predict latsa, latent(eta2)
gen rrm =  cops – copsa
summarize rrm
```

MPLUS CODE

SEM INSTRUCTIONS FOR FEMALE CHOICE MODEL

```
TITLE: SEM with latent variables
ASST  y1
TotS  y2
LA1   y3
LA2   y4
dom   y5
ds    y6
```

```
HST    y7
HS     y8
CopS   y9
CourtS y11;
DATA:
        FILE = C:/Users/Desktop/Mplus/datiSEM1.csv;
VARIABLE:
        NAMES = y1-y8 u9-u10 y11;
        USEVARIABLES=  y1 y2 y3 y4 y7 u9 y11;
  ANALYSIS:
        TYPE=GENERAL;
        ESTIMATOR=MLR;
        STARTS=5;
  MODEL:
        !* The metric of the factor is defined by fixing the factor
variance at 1*!
        f3 by  y7* u9 y11;
        f3@1;
        f2 by y3* y4;
        f2@1;
        f1 by y1 y2;
        f1@1;
        f2 ON f1;
        f3 ON f2;
  MODEL INDIRECT:
        y1 IND f1;
        y2 IND f1;
        y3 IND f2;
        y4 IND f2;
        y7 IND f3;
        u9 IND f3;
        y11 IND f3;
        y7 IND f1;
        u9 IND f1;
        y11 IND f1;
        y7 IND f2;
        u9 IND f2;
        y11 IND f2;
        y3 IND f1;
        y4 IND f1;
        f2 IND f1;
        f3 IND f2;
        f3 IND f1;
  OUTPUT:   TECH1  TECH3  TECH4
```

```
        SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
  SAVEDATA:
    FILE IS DATISEMFCH.TXT;
         PLOT:  TYPE=PLOT3;
```

SEM INSTRUCTIONS FOR DOMINANCE MALE MODEL

```
TITLE: SEM with latent variables
DATA:
        FILE = C:/Users/Desktop/Mplus/datiSEM1.csv;
VARIABLE:
        NAMES = y1-y8 u9-u10 y11;
        USEVARIABLES= y3 y4 y5 y6 y7 u9 y11;
  ANALYSIS:
        TYPE=GENERAL;
        ESTIMATOR=MLR;
        STARTS=5;
  MODEL:
        !* The metric of the factor is defined by fixing the factor
variance at 1*!
        f3 by  y7 u9 y11;
        f3@1;
        f2 by y3 y4;
        f2@1;
        f1a by y5 y6;
        f1a@1;
        f2 ON f1a;
        f3 ON f2;
   MODEL INDIRECT:
        y5 IND f1a;
        y6 IND f1a;
        y3 IND f2;
        y4 IND f2;
        y7 IND f3;
        u9 IND f3;
        y11 IND f3;
        y7 IND f1a;
        u9 IND f1a;
        y11 IND f1a;
        y7 IND f2;
        u9 IND f2;
        y11 IND f2;
        y3 IND f1a;
        y4 IND f1a;
        f2 IND f1a;
```

```
        f3 IND f2;
        f3 IND f1a;
  OUTPUT:   TECH1  TECH3   TECH4
        SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
  SAVEDATA:
    FILE IS DATISEMMDH.TXT;
PLOT:  TYPE=PLOT3;
```

GSEM INSTRUCTIONS FOR FEMALE CHOICE MODEL

```
  TITLE: GSEM with latent variables
DATA:
        FILE = C:/Users/Desktop/Mplus/datiSEM1.csv;
VARIABLE:
        NAMES = y1-y8 u9-u10 y11;
        USEVARIABLES= y1 y2 y3 y4 y7 u9 y11;
        COUNT= u9 (p);
  ANALYSIS:
        INTEGRATION=30;
        TYPE=GENERAL;
        MCONVERGENCE=0.01;
        ESTIMATOR=MLR;
        STARTS=5;
  MODEL:
 !* Measurement Model*!
        f3 by  y7* u9 y11;
        f3@1; !* The metric of the factor is defined by fixing the
factor variance at 1*!
        f2 by y3* y4;
        f2@1;
        f1 by y1 y2;
        f1@1;
!* Structural Model*!
        f2 ON f1;
        f3 ON f2;
 MODEL INDIRECT: !* Total, direct and indirect causal effects*!
        y1 IND f1;
        y2 IND f1;
        y3 IND f2;
        y4 IND f2;
        y7 IND f3;
        u9 IND f3;
        y11 IND f3;
        y7 IND f1;
```

```
        u9 IND f1;
        y11 IND f1;
        y7 IND f2;
        u9 IND f2;
        y11 IND f2;
        y3 IND f1;
        y4 IND f1;
        f2 IND f1;
        f3 IND f2;
        f3 IND f1;
  OUTPUT:   TECH1  TECH3  TECH8 TECH4 TECH10
        SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
  SAVEDATA:
   FILE IS DATIGSEMFCH.TXT;
                PLOT:  TYPE=PLOT3;
```

GSEM INSTRUCTIONS FOR DOMINANCE MALE MODEL

```
  TITLE: GSEM with latent variables
DATA:
        FILE = C:/Users/Desktop/Mplus/datiSEM1.csv;
VARIABLE:
        NAMES = y1-y8 u9-u10 y11;
        USEVARIABLES= y3 y4 y5 y6 y7 u9 y11;
        COUNT= u9 (p);
  ANALYSIS:
        INTEGRATION=30;
        TYPE=GENERAL;
        MCONVERGENCE=0.01;
        ESTIMATOR=MLR;
        STARTS=5;
  MODEL:
        f3 by  y7 u9 y11;
        f3@1; !* The metric of the factor is defined by fixing the
factor variance at 1*!
        f2 by y3 y4;
        f2@1;
        f1a by y5 y6 ;
        f1a@1;
        f2 ON f1a;
        f3 ON f2;
   MODEL INDIRECT:
        y5 IND f1a;
        y6 IND f1a;
        y3 IND f2;
```

```
        y4 IND f2;
        y7 IND f3;
        u9 IND f3;
        y11 IND f3;
        y7 IND f1a;
        u9 IND f1a;
        y11 IND f1a;
        y7 IND f2;
        u9 IND f2;
        y11 IND f2;
        y3 IND f1a;
        y4 IND f1a;
        f2 IND f1a;
        f3 IND f2;
        f3 IND f1a;
OUTPUT:   TECH1  TECH3  TECH8 TECH4 TECH10
        SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
SAVEDATA:
  FILE IS DATIGSEMMDH.TXT;
        PLOT:  TYPE=PLOT3;
```

S4 Table
Summary results models (LM/GLM) analysis

Model	**Type response**	**Predictor variables**							
		ASS_T	*TotS*	*Dom*	*Ds*	LA_1	LA_2	*HS*	*CourtS*
LM_1	Gaussian	-0.097	-0.059	-0.268	0.064	0.146	-0.026	**1.573**	**1.328**
$LM_{1,r}$	Gaussian							**1.584**	**1.480**
LM_2	Gaussian	0.003	-0.010	-0.017	0.003	0.024	-0.004	**0.158**	**0.587**
$LM_{2,r}$	Gaussian							**0.164**	**0.606**
LM_3	Gaussian	-0.006	-0.012	-0.015	0.002	0.027	-0.004	**0.182**	**0.818**
$LM_{3,r}$	Gaussian							**0.188**	**0.839**
LM_4	Gaussian	-0.019	-0.014	-0.008	-0.001	0.031	-0.003	**0.226**	**1.450**
$LM_{4,r}$	Gaussian							**0.231**	**1.473**
LM_5	Gaussian	0.010	-0.015	-0.033	0.006	0.035	-0.006	**0.263**	**0.734**
$LM_{5,r}$	Gaussian							**0.261**	**0.778**
GLM_1	Poisson	0.051	**-0.066**	0.398×10^{-5}	**-0.029**	**0.070**	-0.079	**0.402**	**1.476**
$GLM_{1,r}$	Poisson		**-0.061**		**-0.030**	**0.046**		**0.414**	**1.460**
GLM_2	Neg. Binom.	-0.014	-0.033	-0.016	-0.014	0.076	-0.008	**0.436**	**1.851**
$GLM_{2,r}$	Neg. Binom.							**0.448**	**1.897**
GLM_3	ZIP	0.051	**-0.066**	0.150×10^{-4}	**-0.029**	**0.070**	-0.079	**0.402**	**1.476**
$GLM_{3,r}$	ZIP		**-0.061**		**-0.030**	**0.046**		**0.414**	**1.460**
GLM_4	ZINB	-0.014	-0.033	-0.016	-0.014	0.076	-0.008	**0.436**	**1.851**
$GLM_{4,r}$	ZINB							**0.448**	**1.897**
GLM_5	Hurdle	**0.065**	**-0.054**	-0.046	-0.004	0.043	0.000	**0.335**	0.282
$GLM_{5,r}$	Hurdle	**0.068**	**-0.025**					**0.321**	

Reported values are the unstandardized estimated coefficients. Significant coefficients ($P<0.05$) are shown in bold. Variable names are: ASS_T = the fluctuating asymmetry of small antler's spellers*;* *TotS* = total number of small and large antler's spellers*;* *Dom* = Dominance Index (Clutton-Brock et al. 1979) divided by the total number of bucks of each year; *Ds* = the David's score (Gammel et al. 2003) divided for the total number of bucks of each year; LA_1=number of days in which the animal was present in the lek. LA_2= total number of days of presence/territory in different locations of the same lek. *HS* = average number of females in a male's territory; *CourtS* = the fraction of courtship events terminated with a copulation (number of copulations / number of courtship events, for every male). Response variable is *CopS* = total copulatory success of the i-th buck in one rut. The number of observations is the same for all models (N=118).

References

Clutton-Brock TH, Albon SD, Gibson RM & Guinness FE. (1979) The logical stag: adaptative aspects of fighting in red deer (Cervus elaphus L.). *Animal behaviour*, 27, 211-225.

Gammel MP, De Vries H, Jennings DJ, Carlin CM, Hyden TJ. (2003) David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal Behaviour*, 66, 601-605.

S5 Text
Log-Likelihood of discrete and continuous distributions to calculate AIC, BIC and R code

The Information criterion AIC or BIC provides a way to compare nested and non-nested models. The AIC is computed using the formula AIC=-2log(L)+2q, while BIC = -2log(L)+qlog(n) where L is the likelihood, q the number of estimated parameters in the model and n is the number of observations. The log-likelihoods obtained from a model fitted using a discrete or a transformed response variables are, however, not comparable. A solution is provided by the formula suggest by Weiss (2010), which allows to compare models using an untrasformed and a log-transformed response variable:

$$\mathrm{L(y)} = \prod_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{dnorm}(\mathrm{logy_i};\ \mu_\mathrm{i}, \sigma)\frac{1}{\mathrm{y_i}}$$

or in terms of log-likelihood:

$$\mathrm{logL(y)} = \sum_{\mathrm{i=1}}^{\mathrm{n}} \log\left[\mathrm{dnorm}(\mathrm{logy_i}; \mu_\mathrm{i}\ ; \sigma)\frac{1}{\mathrm{y_i}}\right]$$

or in terms of log-likelihood for square root transformed response model:

$$\mathrm{logL(y)} = \sum_{\mathrm{i=1}}^{\mathrm{n}} \log\left[\mathrm{dnorm}(\sqrt{\mathrm{y_i}}; \mu_\mathrm{i}\ ; \sigma)\frac{1}{2\sqrt{\mathrm{y_i}+0.5}}\right]$$

where dnorm is the density of normal distribution, yi= CopSi, μ_i is the log(CopSi+ei) predicted by the regression model, ei=1,0.5,0.01, $\sqrt{\mathrm{y_i}}$=square root transformed CopSi, s= generate using maximum likelihood estimate from fitting the model to the log transformed response or to the root square transformed response.

R code to calculate a function obtain normal log-likelihood for untrasformed response:

```
norm.test<-function(model,y){
s<-sqrt(sum(residuals(model)^2/length(residuals(model)))
LL<-sum(log(dnorm(y,mean=predict(model),sd=s)))}
norm.test<-(model,y)
```

R code to calculate a function obtain lognormal log-likelihood for trasformed response:

```
norm.log<-function(model,y){
t.y<-log(y+e)
s<-sqrt(sum(residuals(model)^2/length(residuals(model)))
LL<-sum(log(dnorm(t.y,mean=predict(model),sd=s)*1/y))}
norm.log<-(model,y)
```

R code to calculate a function obtain a square root log-likelihood for trasformed response:

```
norm.sqrt<-function(model,y){
t.y<-sqrt(y)
s<-sqrt(sum(residuals(model)^2/length(residuals(model)))
LL<-
sum(log(dnorm(t.y,mean=predict(model),sd=s)*1/(2sqrt(y+0.5))))
}
norm.sqrt<-(model,y)
```

Data set is available in S1 Dataset

Reference

Weiss J. (2010) Statistical Methods in Ecology. University of North Caroline. http://www.unc.edu/courses/2010fall/ecol/563/001/docs/lectures/lecture15.htm [accessed 24 May 2016].

S5 Table
Variance-covariance and correlation matrix used in SEM and GSEM for FCH and MDH models.

S5 Table. Measured covariances (upper triangle), variances (diagonal show in bold type face) and correlations matrix (lower triangle)in a data set consisting of 118 observations. a) FCH model; b) MDH model.

a)

Variables	ASS_T	*TotS*	LA_1	LA_2	*HS*	*CourtS*	*CopS*
ASS_T	**4.094**	0.099	0.191	0.214	0.100	0.103	0.061
TotS	1.248	**39.072**	0.254	0.251	-0.106	0.027	-0.086
LA_1	2.859	11.777	**54.819**	0.907	0.118	0.264	0.234
LA_2	9.103	32.985	141.255	**442.782**	0.067	0.295	0.185
HS	0.314	-1.032	1.356	2.182	**2.410**	0.530	0.627
CourtS	0.208	0.170	1.950	6.189	0.820	**0.992**	0.537
CopS	0.640	-2.774	8.952	20.090	5.031	2.766	**26.717**

b)

Variables	*Ds*	*Dom*	LA_1	LA_2	*HS*	*CourtS*	*CopS*
Ds	**70.847**	0.588	0.154	0.119	0.256	0.256	0.225
Dom	13.008	**6.909**	-0.043	-0.048	0.215	0.104	0.053
LA_1	9.585	-0.831	**54.819**	0.907	0.118	0.264	0.234
LA_2	21.129	-2.656	141.255	**442.782**	0.067	0.295	0.185
HS	3.346	0.879	1.356	2.182	**2.410**	0.530	0.627
CourtS	2.148	0.271	1.950	6.189	0.820	**0.992**	0.537
CopS	9.775	0.713	8.952	20.090	5.031	2.766	**26.717**

S6 Table
The results of a comparison of residuals statistical analysis for LMs with transformed response variable.

S6 Table. Mean, variance, and kurtosis, for residuals distributions of linear models with transformed response variable (x=CopS).

Model	*Transformation*	*Mean*	*Var*	*Kurtosis*
LM_2	*log(x+1)*	0.56	15.15	42.18
$LM_{2,r}$	*log(x+1)*	0.59	16.12	42.06
LM_3	*log(x+0.5)*	0.60	15.59	40.49
$LM_{3,r}$	*log(x+0.5)*	0.63	16.54	40.82
LM_4	*log(x+0.1)*	0.60	16.95	33.53
$LM_{4,r}$	*log(x+0.1)*	0.62	17.79	36.69
LM_5	$x^{0.5}$	0.34	15.53	41.05
$LM_{5,r}$	$x^{0.5}$	0.37	14.79	40.74

S6 Text

Template of data set.

List of data set to implement SEM and GSEM with STATA and Mplus.

For STATA and Mplus will import the data-set: datiSEM_def.xlsx (in S1 Dataset).

CHAPTER 2: *Sexual choice in lekking fallow deer (Dama dama): female mating tactics in relation to costs and experience*

Most studies on fallow deer reproduction focused on the covariates of male reproductive success, while there is much less information on female tactics of mate choice. The aim of this work is to fill this gap and to assess condition-dependent variations in female tactics in the lekking population of Castelporziano (Italy). In particular, we investigated three indirect selection mechanisms: i) *aggregation*, when females join an already formed group; ii) *copying*, when females copy the mate choice of other females and iii) *territory choice*, when females select a territory where many copulations had occurred previously. Our results show that female fallow deer which are less experienced (young) and/or incur in higher travel costs (with a home range far from the lek), adopt indirect forms of mate selection more often than adult females or females residing near the lek. In particular, younger females remained longer in the lek and in the vicinity of bucks than adult ones, and returned to the lek after copulation. However, despite the time spent at the lek, young females were not able to select highest-rank bucks, and relied on *territory choice* more often than adult does. Farther females visited the lek less frequently and arrived later than near females, but they were seen more often within female groups. Surprisingly, we did not find a different amount of *copying* in young or in farther females. Our results can contribute to clarifying the co-evolution of mating strategies of both sexes in ungulate leks.

Introduction

As already noted by Darwin (1871) sexual selection is a fundamental evolutionary force which operates (i) through competition between individuals for access to breeding partners, a process which in males leads to the evolution of different forms of weaponry and fighting ability, and (ii) through female mate selection, leading to the evolution of various forms of exaggerated and costly ornamentation (the peakock’s tail) in males, honest signals of their genetic quality (Andersson, 1994; Davies et al. 2012).

Sexual selection attains extreme levels in lek breeding where many males and females congregate in a small area for mating, a situation that leads to fierce competition. In leks, males aggregate and defend small display territories located very close to one another. This reproductive system is characterised by a strong asymmetry in male reproductive success (Wiley, 1991; Alatalo et al. 1992; Höglund & Alatalo 1995; Alberts et al, 2003) that, in the absence of sexual coercion, is determined by a consensus of female choice. In leks, females do not get any resource from males except genes, even if some authors have argued that females may obtain direct benefits, such as the reduction of transmission of venereal diseases and ectoparasites, or the reduction of social interference (Bradbury 1981; Clutton-Brock et al. 1993; Hoglund & Alatalo, 1995; Apollonio et al. 2014). The ability to compare many males on the same stage leads females to visit these aggregations (Bradbury, 1981). Female choice seems to play an important role in the evolution of the lek and, for the understanding of these processes it is essential to identify which are the costs and benefits for females (Wrangham, 1980; Pomiankowski, 1987; Reynolds and Gross, 1990; Clutton-Brock et al., 1993).

A small number of ungulates are known to form leks such as the topi (*Damaliscus lunatus* Bro-Jørgensen 2002; the sika deer (*Cervus nippon*, Bartos et al. 2003), the Uganda kob (*Kobus kob thomasi*, Deutsch 1994), the white-eared kob (*K. k. leucotis*, Fryxell 1987) and the Kafue lechwe (*K. leche kafuensis*, Nefdt 1995). The fallow deer (*Dama dama*) is an ideal species to study lek mating given that this cervid forms large aggregations, individual behaviour can be easily recorded and males are easily identifiable by their palmed antlers in the absence of tags. Most of previous field studies concerning leks of fallow deer focused on the covariates of male reproductive success (see Lombardi et al. 2017 and references therein), while there is much less information on female tactics of mate choice (Clutton-Brock et al. 1989, Apollonio et al. 2014; for non-lekking populations and controlled experiments see also McComb and Clutton-Brock 1994, Komers et al. 1999, Farrell et al. 2011, Briefer et al. 2013, Naulty et al. 2013;). Our paper aims to fill this gap of knowledge using the availability of information on the behaviour of a number of individually-marked females in the lek of Castelporziano (Italy) (Fig. 1). In particular, the aim of this work is to verify whether or not variable reproductive costs and/or experience of does are associated with different tactics of mate choice.

High costs for lekking females can be expected because of (i) increased energetic expenditure to get to the lek; (ii) increased predation (or accident) risks during the displacement to and from the lek; (iii) less time left for other activities, such as foraging (Gibson and Bachman, 1992). In this study we overlook the analysis of predation risk because large predators were absent in the study area at the time of the study. Energy expenditure and accident risks are plausibly correlated to the distance between one female's home range and the lek, while the permanence in the lek (when located outside a female's home range) is probably correlated to the cost of missed opportunities.

We can safely assume that female experience improves with age only if a doe mates in the lek for several consecutive years. Accordingly, we investigated female mate tactics in relation to age.

As stated above, an important motivation for a female to visit a lek is the possibility of choosing an appropriate mate from among a large number of suitors. A female makes a direct assessment when, after having visited all or most males, chooses one of them exclusively on the basis of his phenotype (Janetos 1980; Andersson and Simmons 2006). Although this behaviour can be quite effective, it requires considerable effort and discerning skills by females. On the contrary, secondary tactics of mate assessment occur when a female choses a partner using cues other than male phenotypic traits. This approach, described in several lekking species (Gibson and Höglund, 1992; Fiske et al., 1996; Kokko 1999), may allow for an efficient choice at reduced costs. Importantly, secondary tactics have the potential to increase the asymmetry observed in male reproductive success (Wade and Pruett-Jones 1990; Alonzo 2008).

In this study we want to assess whether females are more likely to make use of secondary strategies in relation to both the location of their home range with respect to the lek (displacement costs) and their age (experience). We investigated three secondary tactics:

1. *Aggregation*. Clutton-Brock *et al*., (1989) found that the number of females increases in territories where a harem is already present: females could aggregate in order to have higher probabilities to join a successful male.
2. *Copying*. A female mates with a male that was previously observed to mate with other females (Bradbury and Gibson, 1983);
3. T*erritory choice.* A female chooses a male that is defending a territory where other copulations have taken place earlier (Gibson, 1992).

A main difference between *copying* and *territory choice* is that the latter can be based also on indirect cues, such as pheromones or territorial marks, while copying relies only on the direct observation of copulations. *Aggregation* differs from *copying* because it may allow females to have a quick idea about the position of successful males in the lek, even in absence of observed copulations. We formulated a set of six (not mutually exclusive) hypotheses:

H1. Females with no or reduced travel costs should adopt more often a direct assessment tactic than females living far away. We therefore expect that a) the time spent at lek, the number of visits to the lek, and the date of arrival are inversely dependent on the distance between a female home range and the lek. More specifically, we predict that females using a direct choice tactic are present in the lek before the onset of the oestrus in order to observe displaying bucks and evaluate their quality. Consequently, we also expect these females (b) to visit a higher number of territories and to spend more time in the vicinity of bucks.

H2. Secondary tactics allow reduced mating costs for females (Fiske *et al.*, 1996; Kokko et al. 1999). We thus expect that females with higher travel costs would be prone to use secondary mating tactics and, more specifically, we expect that these females are more likely to be (a) observed within female clusters (*aggregation*), and/or (b) to mate with a buck that copulated immediately before her own copulation (*copying*) or (c) mating in a territory where more copulations had occurred earlier (*territory choice*).

H3. Secondary strategies could be used by less experienced (younger) females that are not able to make a good choice of their own. In this case, predictions H2a-2c should be confirmed in young females independent of the distance of their home range from the lek (H3a-3c). We also expect that younger females (d) would stay in the lek longer than adult ones to observe mating behaviour and, consequently, (e) to spend more time in territories and in the vicinity of bucks. This makes sense if they visit the lek when the mating activity is well developed and so we do not expect (f) that arrive at the lek before adult females.

H4. Females using *copying* and *territory choice* (a) should mate later than the ones that make a direct assessment and, consequently, (b) we expect that the asymmetry in male mating success increase over the course of the mating season.

H5. Females do not need to spend any time at lek after mating, except if this is useful for gaining experience. Thus we expect that young females stay longer in the lek than adult ones after their own mating, irrespective of the distance lek-home range.

H6. Secondary tactics could increase the precision in mate assessment (Balmford 1991; Gibson and Hoglund 1992). If this holds, females that follow these tactics should make a better choice than the ones that make a direct assessment. We thus expect (a) highly successful males to be more likely to mate with females that adopt a secondary tactic. We are allowed to use the total mating success of a buck as a proxy for its quality, since Losey et al. (1986) theoretically demonstrated that at least 40% of females should adopt a direct choice behaviour, and that direct choice is on average correct, in order for copying to be an evolutionary stable strategy. Alternatively (b) we can hypothesize that females that make a direct assessment then mate with males that are, on average, of higher quality which results in a high-cost but high-precision female tactic.

Figure 1. A group of female fallow deer (Dama dama) in the lek of Castelporziano (Italy).

MATERIALS AND METHODS

Study area

The study was performed at Castelporziano in the years 2000-2003. The study area is a fenced area of 60 km^2 located near Rome (Italy) (41° 44'N, 12° 24'E) (Fig. S1 in Chapter 1). The climate is Mediterranean, with dry summers and rainfall occurring primarily in October-November. The plant communities mainly consist of holly oak (*Quercus ilex*, 27%) and deciduous oak forests (*Q. cerris* and *Q. frainetto*, 34%), often associated with undergrowth of *Carpinus orientalis* (80-90%). A number of domestic pine (*Pinus pinea*) stands were also present. A very detailed description of the vegetation of the study area can be found in Bianco et al. (2001). Information on ungulate populations are found in Focardi *et al.* (2015).

The lek in Castelporziano Preserve is only one and was located in an open area of about 0.7 km^2, characterised by the presence of an arid pasture. This grassland is characterised by the presence of a few large oaks and a sparse undergrowth with common hawthorn, *Crataegus monogyna*, ferns *Pteridium aquilinum* and *Asphodelum microcarpus* (*=aestivus*). The area lacked large predators, and culling was forbidden during the rut. Rangers reported that fallow have formed a lek in this area at least since the early 80s.

Data collection

We performed animal handling according to the present regulations related to animal welfare and with constant veterinary assistance. Authorisation to capture was made according to act. 4 of the Italian act 157/92. Upon capture, all animals were fitted with a soft mask (to reduce distress), which allowed deer to breathe normally. Deer were positioned on the right side to avoid ruminal meteorism, which might reduce respiratory efficiency. Neonates were handled wearing gloves. No sedation was used and handling time was the shortest possible.

Several methods of capture were used. We carried out 24 net drives during winters (January-February) 2000-2002. Moreover, during the annual trapping season of wild boar, in August-September 1996-2002, some fallow deer (mainly 3–4-month old individuals) were captured incidentally. A small sample of neonates was captured in June 2000-2003 and marked with ear tags. A total of 123 females were ear-tagged (75 of which were observed, at least once, at lek as yearlings or adults in 2000-2003) and 9 adult females were also fitted with VHF radio-collars (Wildlife Materials HPLM 21100, Murphysboro, IL, USA).

Radio-tracking was performed using ATS Rx1000s (Isanti, MN, USA) and Lotek Suretrack 2000 (Newmarket, Ontario, Canada) receivers with a three-element Yagi antenna. Outside the rut, we collected a fix every 24-48 hours (at least 12 fixes per month, homogenously distributed over day and night) while during the rut we increased the sampling rate. Depending on the available personnel, a different sampling design was adopted during the different years of study. In 2000, we took about 1 fix every four hours, with a higher frequency when the animal was moving. In 2001 we took a fix every 12-13 hours, if animals remained in the same area, but we used a four-hour schedule if the animal moved away from the previous location. Finally, in 2002 and 2003 fixes were taken every 24-25 hours but, using a non-directional antenna, we also recorded every three hours the arrival/departure of animals to/from the lek and in this case one or more supplementary fixes were taken.

Out of 75 females observed in the lek (Figure 1), 42 (captured as fawns or yearlings) were of known age. Note that all radio-tracked females were of unknown age. We classified does of known age as: yearlings (15-16 month old) and prime-aged adults (from 27-28 to 87-88 months), indicated for short as 1.5, 2.5,….7.5 years (we refer to this classification as *Age7* with seven age classes). When we also included in the analysis also the does of unknown age, we used three different classifications, depending on the analysis (see below):

1. Yearlings and adults (referred to as *Age2A;* two age classes).
2. Yearlings + young adults (2.5 years old), other adults (*Age2B;* two age classes).
3. Yearlings, young adults (2.5 years old), other adults (*Age3;* three age classes).

For each marked animal we computed the distance between the centre of its home range (outside the rut) and the lek and we considered this value as a proxy for costs borne by females to mate in the lek (Figure 2).

The centre of the home range in spring-summer (immediately before the onset of the rut of the same year) was computed using the best available information. When possible we computed a home range centre for each year. For radio-marked animals we computed the barycenter of March-August fixes. For ear-tagged does we used as proxy of home range centre the barycenter of available observations. Observations were collected mainly during spring counts (April, Focardi et al. 2013), and summer counts of wild boar (July-August, Focardi et al. 2008). When the observations of the same year were not available, we considered the last observation available or the capture location. We are allowed to assume a strict philopatry of does because the radio-tracking data have shown only a single significant shift of the spring-summer home range between two years. We also showed that yearling females almost never left the maternal home range (Imperio et al. in prep.). Because of the limited precision of the computed distances we approximated these value using 1 km classes of distance binned from 0.5 to 7.5 km (*Dist*).

During the rut a variable number (1 to 4) of operators observed the behaviour of fallow deer at the lek. The observers were equipped with binoculars (*Zeiss 10x40, 20x80 Vixen* and *30x75 Swarovski*) and telescopes (*Swarovski 60x*) to be able to read the tags. To check most of the lek area operators used a blind and/or a high seat depending on the position of display territories. Observations were performed *ad libitum* from dawn to dusk.

The survey performed in 2000 has to be considered a pilot study since observations lasted from the 1st to 16th of October and only the presence of tagged animals and the number of copulations were recorded.

In 2001-2003 a standardized sampling protocol was adopted. Behavioural observations were carried out beginning the 20th of September until the end of October. The arrival of the first female groups in the lek was considered as the starting date of the rut. The end date was instead identified as the day after the last observed copulation. The great majority of bucks holding a lek territory were individually identified by antler morphology (Lombardi et al. 2017). Each hour we recorded the position and sex/age class of every animal inside the lek (hourly counts). The location and the association of tagged females with territory holders or other females were recorded in 2001 as often as possible, depending opportunistically on available observers and tasks to be performed, while in 2002-2003 we tried to record females' movement continuously. We noted territory and buck identity of all copulations of both tagged and non-tagged females.

Every year we recorded the daily number of copulations and we distinguished between courtships ending with ejaculation (*Ejac*) and interrupted courtships (*IntC*). We used the median date of copulation as a measure of copulatory peak (*Peak*), and we divided the rut into *pre-Peak* and *post-Peak* periods.

Variables	***Descriptions***	***Sampling years***	***Selection criterion***
Cop_preP	Number of copulations before *Peak*	2001-2003	≥5 obs
Cop_postP	Number of copulations after *Peak*	2001-2003	≥5 obs
Visits	Number of visits of female at lek	2000-2003	
Date_arr	Date of first arrival at lek (days from *Peak*)	2000-2003	
Time_lek	Time (hours) spent in the lek	2000-2003	
Time_lek_ct	Corrected time spent in the lek (hours)	2000-2003	≥1 hour
Date_cop	Date of copulation (days from *Peak*)	2001-2003	*Ejac*
Visits_after	Number of visits at lek for females after copulation	2001-2003	*Ejac*
N_Terr	Number of territories visited by a female	2002-2003	≥3 min
Time_terr	Total time (min) spent in display territories	2002-2003	≥3 min
Time_buck	Total time (min) spent with bucks	2002-2003	≥1 min
P_Cluster	Probability to be observed together with other females	2001-2003	≥2 obs
Clust	Binary variable: 0= alone, 1=in group of females	2001-2003	1st obs
Male_cop_day	Number of copulations of the buck, with which the female mated, from the dawn of the same day until the mating with the female	2001-2003	*Ejac+IntC*
Male_cop_2day	Number of copulations of the buck, with which the female mated, from the dawn of previous day until the mating with the female	2001-2003	*Ejac+IntC*
Male_cop_before	Number of copulations of the buck, with which the female mated, from the beginning of rut until the mating with the female	2001-2003	*Ejac+IntC*
Terr_cop_day	Number of copulations observed in the territory, where the female mated, from dawn of the same day until the mating with the female	2001-2003	*Ejac+IntC*

Variables	*Descriptions*	*Sampling years*	*Selection criterion*
Terr_cop_2day	Number of copulations observed in the territory, where the female mated, from dawn of previous day until the mating with the female	2001-2003	*Ejac+IntC*
Terr_cop_before	Number of copulations observed in the territory, where the female mated, from the beginning of rut until the mating with the female	2001-2003	*Ejac+IntC*
Male_cop_tot	Total copulatory success of the buck with which the female mated	2001-2003	*Ejac+IntC*

Table 1. Descriptions of dependent variables. We report the years used for computing the different variables and the criterion used for inclusion in the analysis. Obs= observations; Ejac= number of copulations ending with ejaculation; Ejac+IntC=total number of courtships; Peak= peak date.

We also computed the overall rank of each buck and territory from the distribution of copulations recorded for each buck or territory. Specifically, ranks were from 1 to 5 (bins: >22, 10-22, 4-9, 1-3 and 0) for bucks and from 1 to 4 for territories (bins: >10, 6-10, 1-5 and 0). The variables used in the analyses are listed in Table 1, with a brief description for each of them. The scale of the variables is always at level of (female) individual/year except for *Cop_preP* and *Cop_postP,* which are computed at level of (buck) population/year and *Clust* that is computed at level of single observation occasion (of females).

The date of arrival in the lek, *Date_arr* and the number of visits to the lek, *Visits,* were computed using different field data for radio-tracked does and ear-tagged females, censequently are not directly comparable. For ear-tagged females observed at lek, observations in two consecutive days were ascribed to the same visit only if the time lag (in hours) between observations was lower than the average visit duration computed for radio-tracked females. Lek attendance time, *Time_lek* was computed only for radio-tracked does as the sum of time spent in the lek during each visit, including the first and last fixes recorded in the lek. A similar metric was computed for tagged females considering the first and last observation of each visit at the lek. Since observation efforts varied from year to year we corrected this value by multiplying it by the yearly average ratio between *Time_lek* / *Time_lek_obs* (number of observations made during the year) computed for radio-tracked females (*Time_lek_ct= Corrected time spent in the lek*). All other variables were derived from direct observations.

The rationale behind using different metrics for the copulatory success of a buck territory (Table 1) is as follows. Since the average duration of a visit to the lek by an adult female is about two days, if the female uses *copying* or *territory choice* relying on visual cues we expect that the copulation happened on the same day (*Male_cop_day*, *Terr_cop_day*) or in the two last days (*Male_cop_2day, Terr_cop_2day*) are those of interest. If the female uses indirect cues inside the territory, such as marking, we expect that the whole previous period be considered relevant (*Terr_cop_before)*. Moreover, some females could stay longer than average in the lek, therefore both *Terr_cop_before* and *Male_cop_before* could have some importance. Instead, in the case a female mates with a buck on the basis of its physical feis a reliable index of its quality. For these analyses we used both *Ejac* and *IntC* because, irrespective of the outcome, any courtship indicate a mate selection by the female. Note that for variables involving copulations (**_cop* and *Visits_after*) in case of multiple female copulations we used only the first one observed.

Statistical analysis

We compared the variance of *Cop_preP* and *Cop_postP* to investigate whether the unanimity of female choice increased during the rutting season. We selected only bucks observed ≥5 times on lek during the hourly counts to avoid artificially increasing the number of zeros. Variances were compared using the Fisher's test (PROC TTEST of SAS).

The main aim of this study is to verify how the mating tactics of females (described by the variables listed in Table 1) depend on experience (female age) and costs (distance between lek and home range). Since some females were observed in several years we used generalized linear mixed models (GLMM) (Bolker 2015, Zuur et al. 2012) of PROC GLIMMIX of SAS 9.4 (SAS Institute 2012) including female identity as a random factor. Because many dependent variables were not normally-distributed, the appropriate distribution was selected (Zuur et al. 2012).

For *Clust*, i.e the occurrence of finding a newly arrived female in the lek grouped with other females, we used a binary distribution; for this variable only we tested also the effect of the ranks of bucks and territory to disentangle the effect of aggregation among females *per se* and the attractiveness of successful bucks and territories.

Results

Copulatory success of bucks was highly asymmetric and varied from year to year. In 2001, only 54 out of the 133 individually recognised bucks mated at least once, and the first ten most successful bucks performed 49.5% of all the copulations at lek. In 2002, 42 out of 114 bucks mated, and the first ten were responsible for 62.6% of copulations. Finally, in 2003, 77 out of 172 bucks mated, and the first ten performed 50.2% of total copulations. The median date of the distribution of copulations (*Peak*) is reported in Table 2 for each year, together with the variance of the male reproductive success for the pre- and the post-*Peak* periods. It is evident that the variance of male copulatory success was larger in the post-*Peak* period confirming a larger consensus of female choice later in the season. The distribution of male copulatory success is displayed in Fig. S2.

Year	***Ejac***	***Ejac+IntC***	***Peak***	***SD pre-Peak***	***SD post-Peak***	***F***	***p***
2000	116	148	11th Oct	–	–	–	–
2001	244	319	11th Oct	2.88	3.84	1.78	0.007
2002	197	243	13th Oct	3.54	4.94	1.94	0.006
2003	445	638	15th Oct	2.28	4.85	4.51	<0.001

Table 2. Number of copulations ending with ejaculation (Ejac), total number of courtships (Ejac+IntC), Peak date, standard deviation of male copulatory success during the pre-Peak and post-Peak phases, and results of equality of variances test (Fisher's test) (prediction H4b). The test was not performed in 2000 since bucks were not individually identified.

Almost all radio-tracked females visited the lek every year: only the farthest female (distance class: 7.5 km) visited the lek (once) in 2001 and 2003, but not in 2002. The radio-collared females whose home range was closer to the lek visited the lek more frequently (up to 7 visits; Model M1, Table 3), arrived earlier (on average the females of distance class 0.5-1.5 arrived 5 days before than the females of class 6.5-7.5; M2, Table 3) and exhibited a longer overall lek attendance (up to 208 hours; M3, Table 3) than females living farther away. In Fig. S3 we show the distribution of maximum presence per day of does and bucks in the lek from 2001 to 2003. To note that the average length of a single visit (20.46±2.76 hours) was not dependent on the number of visits (Kruskaal-Wallis test: H=5.42, p=0.49). In case of multiple visits, the average interval between successive visits was 4.24±0.42 days

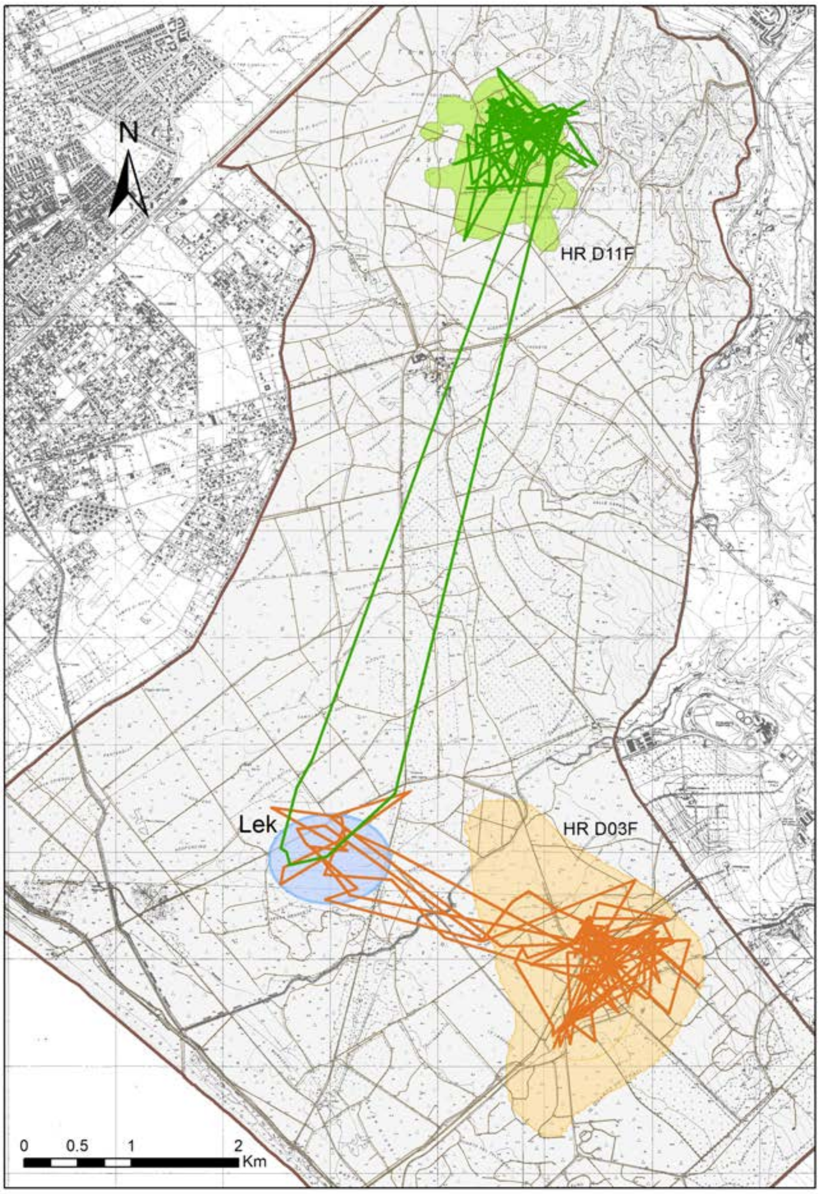


Figure 2. Paths of two radio-collared females (green: D11F, *Dist*=7.5 km; orange: D03F, *Dist*=2.5 km), performing one and three visits to the lek, respectively, during the breeding season in 2000 in the Castelporziano Preserve, Italy. Light orange and green areas represent the spring-summer home ranges of the two females, while the blue ellipse encloses the lek area (see Fig. S1).

Considering the larger sample of all the tagged females, we confirm the previous results relative to radio-tracked does in relation to distance (Models M4-M6, Table 3). From Models M4-M6 we can also deduce that younger females were more prone to visit and stayed longer in the lek than adult ones, but they did not arrive in the lek before adult females (M5, Table 3).

During 2001-2003 we observed the mating of 29 tagged females (plus the interrupted courtships of other 6 does), of which three copulations were with an unknown buck, and two in a unrecorded territory. The large majority of females (90%) mated only once, but two does were seen to mate twice (one yearling in the same day in 2002, and one 2-year old female after 4 days in 2003), and one 2-year old female mated three times in 2003 (the second time after 20 days, third time in the same day), all of them with different bucks. Contrary to our predictions, there was no relation between copulation date and distance, while we found that younger females mated later than adult females (M7, Table 3). If we pool yearlings with 2-year old females, the number of visits in the lek after mating did not depend on the distance but it was higher for the younger class (M8, Table 3). A larger fraction of young females (83.3%) than either 2-year old (50%) or older females (21%) returned to the lek after mating (χ_2^2=7.80, P=0.020).This result remains valid even pooling youngs and subadults to be compared to adults (χ^2=4.75, P=0.029)

If we consider the female behaviour at lek in more detail, we found that younger females visited a higher number of territories (M9, Table 3), spent more time in display territories (M10, Table 3) and in proximity to a territorial buck (M11, Table 3), than adult females. Contrary to our expectations, none of these variables was related to distance (M9-M11, Table 3).

Interestingly, females living far away were more likely to stay in a female group than other females. This effect was not significant for the whole period of lek attendance (M12, Table 3, *p*(*Dist*)=0.08), but it became significant when we considered only the first observation at lek (M13, Table 3). Introducing the effect of the rank of the buck or of the territory did not improve the model (ΔAICc=3.7, ΔAICc=0.7, respectively), and neither of the variables were significant (*p*=0.42 and *p*=0.25, respectively).

The previous sexual performance of the buck chosen by the tagged females did not depend on distance or age class (M14-16, Table 3). The same pattern also arose when considering the mating territory instead of the buck during the same day or from the previous day (M17-M18, Table 3), despite in this latter analysis there was a slight, non-significant, negative effect of age (M18, *p(Age)*=0.10). Instead, the number of copulations recorded in the territory from the beginning of the mating season to the time of copulation depended on age and it was higher for the young females (M19, Table 3; Fig. 3a).

Finally, considering the whole mating season, adult females were able to mate with higher ranking bucks than young females, while there was no effect of distance (M20, Table 3; Fig.3b).

Model	Description	Hp	Type	N	Distance	p(Dist)	Age	p(Age)
M1	*Visits ~ log(Dist)*	H1a	*Poisson*	27	-0.65±0.15	<0.001	-	-
M2	*Date_arr ~ log(Dist)*	H1a	*Normal*	26	3.78±1.10	0.003	-	-
M3	*Time_lek ~ log(Dist)*	H1a	*Lognormal*	26	-076±0.21	0.002	-	-
M4	*Visits ~ log(Dist) + Age7*	H1a, H3d	*Poisson*	79	-0.29±0.10	0.006	-0.17±0.06	0.008
M5	*Date_arr ~ log(Dist) + Age7*	H1a, H3f	*Normal*	79	2.31±1.00	0.03	0.25±0.57	NS
M6	*Time_lek_ct ~ log(Dist) + Age7*	H1a, H3d	*Lognormal*	74	-0.30±0.11	0.01	-0.14±0.06	0.03
M7	*Date_cop ~ log(Dist) + Age3*	H4a	*Normal*	29	-0.73± 1.21	NS	-4.63±0.86	0.03
M8	*Visits_after ~ log(Dist) + Age2B*	H5	*Poisson*	29	0.071±0.30	NS	-0.97±0.30	0.045
M9	*N_Terr ~ log(Dist) + Age7*	H1b, H3e	*Poisson*	55	-0.15±0.13	NS	-0.36±0.07	<0.001
M10	*Time_Terr ~ log(Dist) + Age7*	H1b, H3e	*Normal*	55	-34.2±36.3	NS	-44.3±19.7	0.04
M11	*Time_buck ~ log(Dist) + Age7*	H1b, H3e	*Normal*	59	-12.55±15.82	NS	-20.3±8.63	0.03
M12	*P_cluster ~ log(Dist) + Age2A*	H2a, H3a	*Logit*	111	0.23±0.13	NS	-0.01±0.17	NS
M13	*Clust ~ Dist + Age2A*	H2a, H3a	*Binary*	111	0.24±0.11	0.04	0.76±0.53	NS
M14	*Male_cop_day ~ log(Dist) + Age3*	H2b, H3b	*Poisson*	32	-0.46±0.36	NS	0.33±0.39	NS
M15	*Male_cop_2days ~ log(Dist)+ Age3*	H2b, H3b	*Poisson*	32	-0.43±0.35	NS	0.01±0.33	NS
M16	*Male_cop_before ~ log(Dist) + Age3*	H2b, H3b	*Poisson*	32	-0.34±0.41	NS	0.47±0.31	NS
M17	*Terr_cop_day ~ log(Dist) + Age3*	H2c, H3c	*Poisson*	33	-0.14±0.48	NS	-0.61±0.44	NS
M18	*Terr_cop_2days ~ log(Dist) + Age3*	H2c, H3c	*Poisson*	33	-0.26±0.54	NS	-0.82±0.43	NS
M19	*Terr_cop_before ~ log(Dist) + Age3*	H2c, H3c	*Poisson*	33	0.13±0.35	NS	-1.32±0.21	<0.001
M20	*Male_cop_tot ~ log(Dist) + Age3*	H6	*Poisson*	32	-0.19±0.26	NS	0.84±0.20	0.005

Table 3. Results of Generalised Linear Mixed Models performed to characterise behaviour at lek and mate choice of female fallow deer in relation to age and distance between home range and lek. Codes for hypotheses/predictions follow the list given in the Introduction.

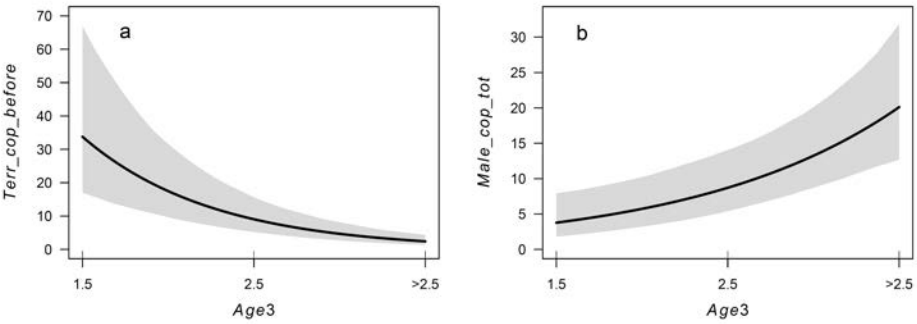


Figure 3. Relationship between age of a female mating in the lek and (a) the number of matings occurred before in the same territory from the beginning of the breeding season (M19, Table 3), and (b) the total copulatory success of the buck with which the female mated (M20, Table 3) in the Castelporziano Preserve, Italy. Confidence intervals of the mixed models were produced using parametric bootstrap, with 500 replicates.

Discussion

Traditionally the analysis of reproductive strategies in ungulates where females do not receive benefits from males, except genes, is focused on male mating tactics (see Lombardi et al. 2017 and references therein) since females are believed to be especially constrained by the resources used for rearing and protecting offspring (Clutton-Brock et al. 1988; Clutton-Brock et al. 1996 ; Ciuti et al. 2006). A small number of papers have dealt with female mating tactics in lekking ungulates (Bro-Jørgensen 2002)

This paper elucidates several open questions which were not dealt with in previous works relative to lek mating. The present analysis showed that female fallow deer adopt different tactics of mate choice in a lek and that variations in the mating tactics are associated with female experience and costs: females, who were either less experienced or incurred higher travel costs more often adopted indirect forms of mate selection when compared to adult females residing near the lek. The large number of visits to the lek recorded in this study for some of the females was never observed before in other fallow deer leks (Apollonio et al. 2014, where however young females were not investigated). This difference may be attributed to: (1) a different study area size (Castelporziano with only one lek is 1.5 times larger than San Rossore where two leks have been observed, Apollonio et al. 2014), (2) hypothetical differences in population density and (3) in male dominance structure which affects the number of female visits (Apollonio et al. 1989).

Visits usually precede the copulation date in particular for adult females. The mean time interval between successive visits is longer than the duration of the oestrus (2 days) but shorter than the oestrus cycle (22 days, Asher, 1985), thus multiple visits likely represent an opportunity for male evaluation rather than mating occasions, as it is argued by Apollonio et al. (2014).

An original finding of this paper is that younger females remained longer in the lek, performed more visits, were observed in a higher number of territories and longer in the vicinity of bucks than adults and returned to the lek also after copulating, fully confirming hypotheses H3d,e and H5. Younger (1-2 year old) females were also the only ones to be recorded copulating more than once, however our limited sample (3 out of 29 tagged females) does not allow us to generalise this observation, and besides, a previous study found that fallow deer polyandry is not related to female age in a non-lekking population (Briefer et al. 2013). Overall, this apparently inefficient behaviour might be a by-product of their lack of experience and thus it can act as a learning occasion. Further, young females have no fawn at heel and their movements are less constrained than the ones of adult does (Ciuti et al. 2006). Multiple visits could favour mate quality assessment (Apollonio et al. 2014), however the young females did not arrive earlier than older ones, albeit males are already present and available to be assessed (cf. Fig. S3) confirming hypothesis H3f. We might therefore deduce that young females are mainly interested in observing the behaviour of other females at lek and in fact they were characterised by a later copulation date, as predicted by H4a. The same was also observed by Farrell et al. (2011) in a non-lekking population. Nevertheless, we found no evidence of a higher probability of copying in young females, contrary to hypothesis H3b. A later copulation date of younger females could be explained by a difference in reproductive physiology between young and adult does (Mattiello 1994), or females of different age might respond differently to social stimuli: females can in fact adjust the timing of oestrus to maximise the possibility to mate with the preferred male (Komers et al. 1999). As a consequence, when compared to adult females, young females could be ready to make their mate choice only after a longer assessment of males at lek than adult females.

Despite the time spent at lek, it appears that young females are not able to select the highest rank bucks, contrary to hypothesis

H6a. Probably younger females are not able to identify high-ranking bucks because of their inexperience in assessing male physical features or in recognizing visual and olfactory marking activities that are supposed to be an important signalling of male reproductive status (Stenstrom et al. 2000). Also in a non-lekking population, younger females were found to mate on average with bucks of lower rank than adult ones (Farell et al. 2011), indicating that this inability of young females is widespread in fallow deer populations regardless the adopted mating system.

Females living far away compensate for higher costs by spending less time, visiting less often and arriving later to the lek than near females, confirming predictions H1a. Despite these constraints, there is no difference between farther and near females with respect to the number of visited territories and time spent in the vicinity of bucks, thus rejecting predictions H1b, as well as in copulation date. Farther females appear to use *aggregation* more than near females, though, as assumed by hypothesis H2a. Experimental studies showed that female fallow deer are attracted un-specifically by female groups (McComb & Clutton-Brock 1994) and these authors argued that this behaviour is probably useful to reduce harassment by immature males and that it allows females to copy the mate choice of others does (Clutton-Brock 1988; Wiley, 1991). However, we did not find evidence for a higher probability of *copying* or *territory choice* in farther females, contrary to predictions H2b-c, therefore *aggregation* could be used as a cheap proxy for high ranking bucks, thus allowing to narrow direct assessment to a lower number of males. The final outcome is that the reproductive success is independent on distance, suggesting that farther females are more efficient than close females, an effect which we did not expect when prediction H6 had been defined. Surprisingly, younger females when compared to older ones, are not more likely to be observed together with other females, contrary to hypothesis H3a. This observation confirms that young females are not as efficient in mate selection as adult does.

The fact that far and near females exhibit the same reproductive success mating with same rank bucks suggest a mechanism for the origin of multiple arenas which are present in some populations (Balmford & Turyhao 1992, Apollonio et al. 2014). As far as the increased costs of a longer distance between the home range and the lek can be buffered by modifications in female behaviour, as we have shown in this paper, the whole population can congregate in a single lek, but we expect that when farther females are no more able to compensate travel costs they are more likely to congregate in a different but nearer arena. We believe that in the case of Castelporziano, we are at the limit of a size where a single lek can be present; indeed the farthest females of our samples were in a distance class of 8.5 km and not every year were able to attain the lek. The critical distance threshold is not expected to be the same in every study area since travel costs depend on a complex way of topography, vegetation type, physical barriers (roads, channels) presence of predators, hunting and disturbance.

Mate choice by females based on the selection of successful territories is widespread in lekking species (fallow deer: Apollonio et al. 1990; Uganda kob *Kobus kob thomasi*: Balmford 1991; blackbuck *Antilope cervicapra*: Isvaran and Jhala 2000; black grouse *Tetrao tetrix*: Kokko et al. 1999; great snipe *Gallinago media*: Sæther et al. 2005). This is because the location of a territory within the lek can be an honest signal of male quality, enabling less costly mate sampling and potentially more accurate mate choice than direct female mate assessment (Kokko et al. 1999), else females may select territories partly using environmental cues, regardless the rank of the male (Sæther et al. 2005). A novel result in our study is that younger females are more likely to select a successful territory (*territory choice*) than older females confirming hypothesis H3c. They could use this tactic to increase the probability of mating with a high quality buck but this study has shown that such an approach is on average unsuccessful, probably because of the rapid turnover of bucks on better territories. Thus, male reproductive variance increases later in the season (hypothesis H4b) can be explained, according to prediction H4a, by young females adopting a tactic of *territory choice* and so mating mostly after the *Peak*.

Contrary to hypotheses H2b and H3b we were unable to detect a different amount of *copying* in young or in farther females. This observation had been unexpected because previous studies have suggested that copying can be an effective tactic for cost-reducing and increasing precision (Gibson and Höglund 1992). This finding concords with McComb and Clutton-Brock, (1994) who found aggregation, but not copying, in female fallow deer under experimental conditions. The review by Vakirtzis (2011) evidences that the presence of copying is a variable in lekking species. Pruett-Jones (1992) used a theoretical game to show that it is convenient for females to copy each other’s mating decisions only when the costs of searching are not negligible. This is not the case of Castelporziano, an undisturbed area with no obstacles to animals’ movement. Moreover, Gibson and Höglund (1992) argued that the benefits of imitation increase if the individuals that mate first are the ones with the greatest experience so we have hypothesized a more frequent *copying* in younger does. Losey et al. (1986) have shown that the benefit of copying increases with the number of “peeks” at lek and indeed young females stay longer at the lek than adults. However, younger females can be deterred to copy mate choice by a rapid male turn-over in territories. On the other hand, farther females cannot spend enough time in the lek to get enough peeks. A further argument which can explain the lack of differences in copying among near and farther females is males' sperm depletion, more likely in bucks who have mated earlier (Gibson and Höglund 1992). Insemination failure is a problem especially for farther females, which would incur in higher costs than near ones if obliged to come back to the lek.

Our results suggest, but do not prove, that adult near females probably perform a direct choice more than farther females. Indeed they stay longer, visit more and arrive earlier as predicted by H1. We can explain this pattern assuming that direct choice is especially complex and requires a careful assessment of both physical features and behaviour of bucks. In other species, direct choice is used more than indirect tactics. This is the case of the great snipe (Sæther et al 2005) and of the non-lekking pied flycatcher *Ficedula hypoleuca* (Slagsvold and Viljugrein 1999). Since fallow deer exhibit a strong plasticity in the mating system (Langbein 1999) which also depends on population density (Apollonio 1989) we can expect that direct choice may vary among populations and be more frequent in low density populations.

This study demonstrated the condition-dependent variation in female mating decisions in an ungulate lek, a crucial issue to improve the understanding of mammalian mating systems (Bro-Jørgensen 2011) and already evidenced in a wide range of taxa (Cotton et al. 2006). Our results can thus contribute to further clarifying the basis of co-evolution of mating strategies in both sexes and, eventually, the evolution of ungulate leks.

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Supporting Information

Fig. S2
Distribution of male copulatory success at lek
in the breeding seasons 2001-2003.

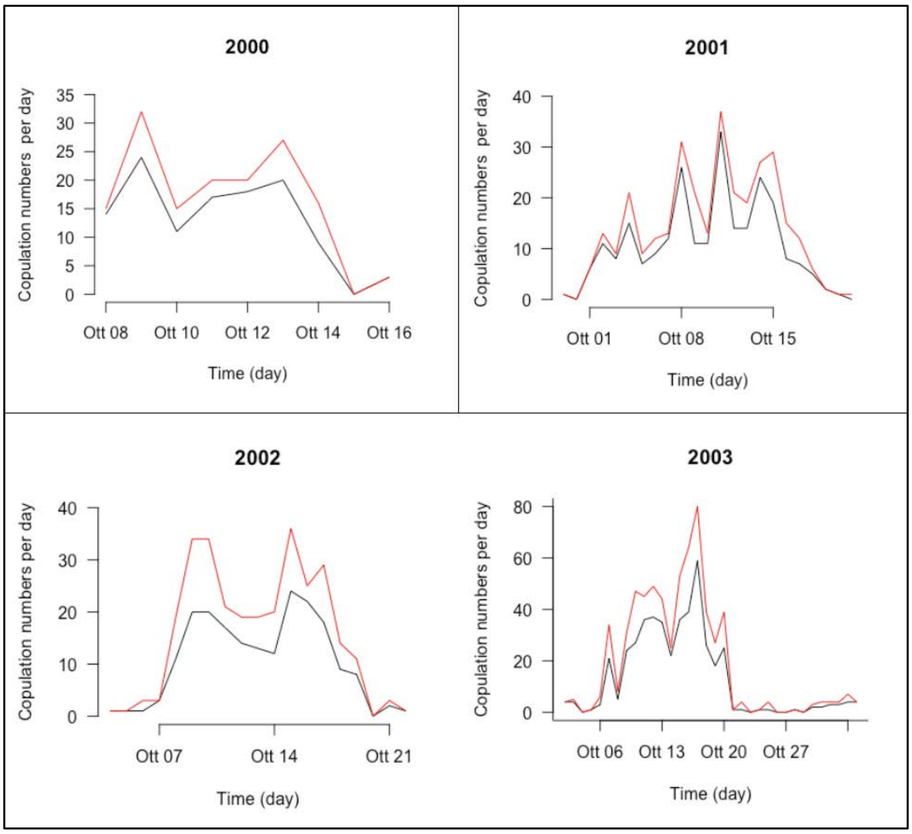


Figure S2. We show the distribution of male copulatory success from 2000 to 2003. The black lines represent the copulation numbers (Ejac) per day;

the red lines represent the total number of courtships (Ejac+IntC) per day; the Peak date per year is showed in Table 2.

Fig. S3

Daily number of does and bucks present in the lek during the breeding seasons 2001-2003.

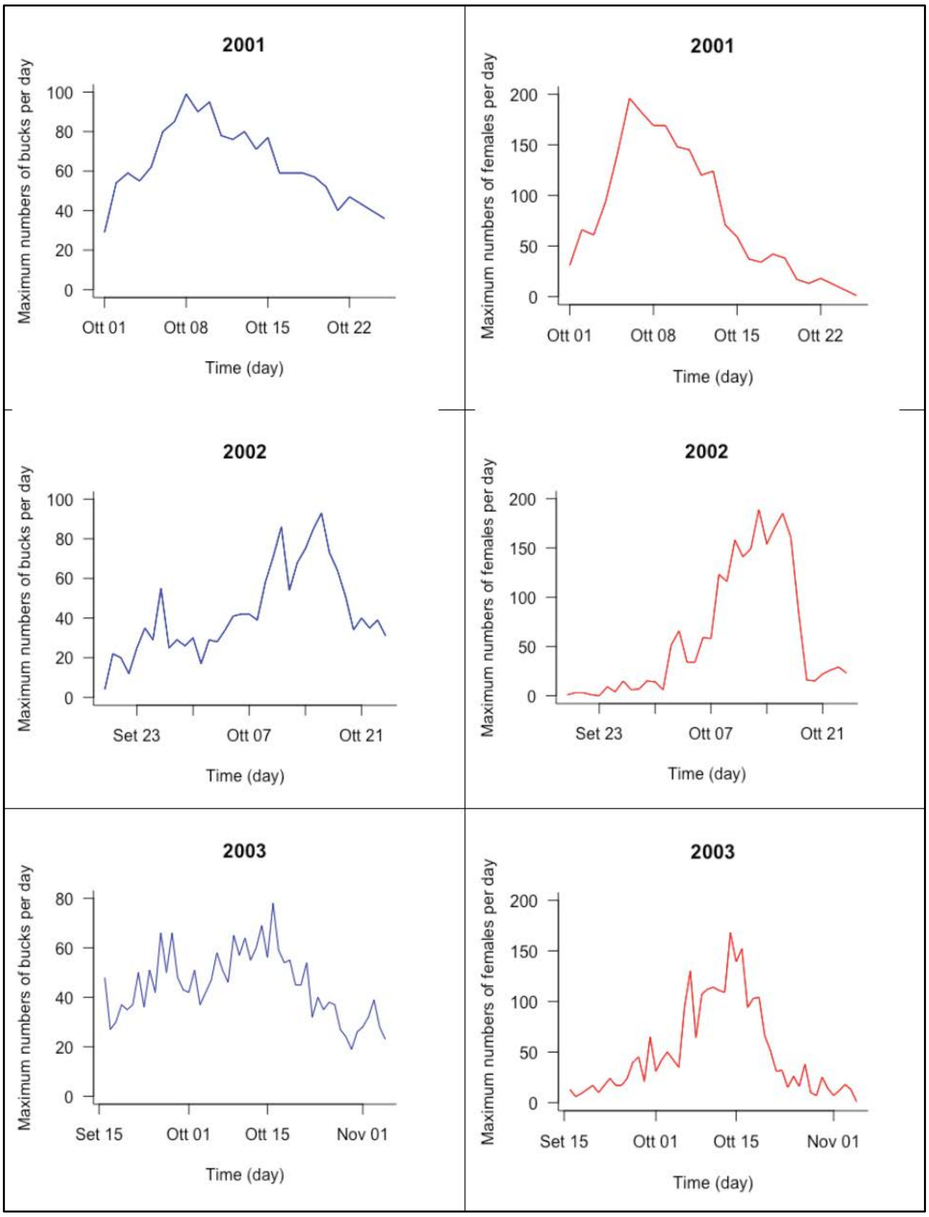


Figure S3. Number of does and bucks present in the lek during the breeding season from 2001 to 2003.

CHAPTER 3: *Generalized structural equations account for ecological complexity in a Mediterranean ungulate community.*

Empirical studies of ecological communities need to account for a large number of reciprocal effects among the components of the studied ecosystem. It is of the uppermost interest to disentangle all these relationships, to discriminate causal effects from spurious correlations and deal with observational errors, which, in studies performed under natural conditions may be large and have the potential to bias our conclusions. To overcome these problems, the use of structural equation models (SEM) is becoming more and more widespread in ecology. Here we review the pertinent literature and discuss pros and cons of this statistical methodology. Further, we show the application of SEM to the analysis of a 100-years-long time series relative to the abundance of three sympatric species of wild ungulates (roe deer, wild boar and fallow deer). Finally, we demonstrate the advantages provided by an innovative methodology (Generalized SEM - GSEM) which has a great potential for analyzing complex networks, where variables are non-normally distributed or categorical. In particular, in this study we tested two main hypotheses: i) the complexity level of the ungulate community affects the strength of intra- and inter-specific competition; ii) intra- and inter-specific interactions are stronger than climate forcing in a Mediterranean environment.

Introduction

Population ecology is the study of how populations change over time and space. The ability to predict population change is fundamental in many different contexts, and it is the basis for a sound management of natural populations. Among the factors recognised as important in affecting population changes, climate and competition play a major role (Putman 2012). Climate is an important determinant of many ecological processes (Stenseth et al. 2002), and both direct and indirect influences on the dynamics of ungulate populations have been described (reviews in Weladji et al. 2002; Mysterud et al. 2003). In North Europe, for example, ungulate population are known to decline after particularly snowy winters (e.g. Jacobson et al. 2004; Grøtan et al. 2005; Mysterud and Østbye 2006b), and negative effects of severe winter conditions on individual body mass (Cederlund et al. 1991) have been described as well. Climate conditions (temperature and precipitation) during early summer, whose effects are mediated by plant growth and availability, are important determinants of individual growth (Langvatn et al. 1996; Mysterud et al. 2001). As all ungulate species (e.g. deer), have a fairly fixed breeding time, changes in environmental conditions may determine a mistmatch between individual needs and food supply, with negative effects on population growth rates (e.g. Plard et al. 2014).

According to Putman (1996) competition is expected to be the most important type of interaction among large herbivores in the absence of large predators. The most frequent type of competition among large herbivores is exploitation competition (Dolman & Wäber 2008), which occurs when the use of a resource by one individual reduces the availability of that resource to another individual. Many studies have suggested that, when resources are limited, the potential for competition is high among sympatric species, as their habitats and nutritional niches often overlap (Bartos et al. 2002). Examples are provided by Bartos et al. (2002) for white-tailed deer Odocoileus virginianus, fallow deer *Dama dama*, red deer *Cervus elaphus*, and roe deer *Capreolus capreolus*; Focardi et al. (2006) for roe deer and fallow deer; Hemami et al. (2004) for roe deer and muntjac *Muntiacus reevesi*; Mysterud et al. (2007) for roe deer and wild boar *Sus scrofa*; Storms et al. (2008) for red deer *Cervus elaphus*, and roe deer *Capreolus capreolus*; Richard et al. (2010) for red deer *Cervus elaphus* and roe deer; Ferretti et al. (2012) for roe deer and fallow deer; Imperio et al. (2012) for roe deer *Capreolus capreolus italicus*, red deer, fallow deer and wild boar *Sus scrofa majori*.

In this paper, we re-analysed the population dynamic of three wild ungulate species in the Castelporziano Preserve near Rome, Italy, as obtained from detailed bag counts from hunting drives during the period 1878–1986: the Italian roe deer *Capreolus capreolus italicus*, the Maremma wild boar *Sus scrofa majori* (both endemic to Italy), and the alien fallow deer *Dama dama*. The native red deer *Cervus elaphus*, is not present today in the Preserve, as it was completely eradicated during WWII (Imperio et al. 2012). Competitive interactions among these species in the Mediterranean area, have been described in a few previous papers. As for example, Focardi et al. (2006), demonstrated that at Castelporziano (Rome, Italy) high densities of fallow deer may reduce habitat quality for roe deer, forcing the latter to achieve smaller body size and larger home ranges. In another area (Maremma Park, Tuscany, Italy), Ferretti et al. (2008, 2011a) documented behavioural interference of fallow to roe deer, showing that fallow deer is able to exclude the roe deer from feeding sites, also using direct aggression (Ferretti, 2011). In both cases, roe deer numbers declined as fallow deer density increased (Ferretti et al., 2011a; Focardi et al., 2006).

The aim of this study is to detect which factors (endogenous and/or exogenous) affect the per capita growth rate r and thus drive population fluctuations. Two main hypotheses have been proposed to explain the population fluctuations in these species: i) the complexity of the ungulate community influences intra- and inter-specific interactions; ii) in Mediterranean environments intra- and inter-specific interactions are stronger than climate forcing.

In our paper we expect negative effects of intra-specific competition and spring-summer drought on the population growth rates of all species, through a negative influence on the fecundity of primiparous females. For polycotous species showing sharp variations in litter size, such as wild boar and roe deer, changes in juvenile survival in summer could be important in determining population fluctuations. Therefore, we can hypothesize a negative influence of adverse weather conditions during the birth season: an excess of rainfall when most of the newborn piglets (in May) or fawns (in May–June for deer species) rest on the ground, could impair thermoregulation determining excess mortality (Van Moorter et al. 2009). Additionally, we also expect that summer drought may negatively impact the growth rate of these ungulate species.

A number of different statistical techniques have been used to investigate inter and intraspecific interactions in ungulates (e.g. Turchin 2003; Putman 2012). Most papers have applied standard linear models, Pearson correlation, Linear and Mixed models to account for repeated observations (Misterud 2007; Richard et al 2010; Ferretti et al. 2012). Few papers have used different approaches, such as path analysis (Misterud et al. 2008) and Structural Equation Modelling (Focardi et al. 2006; Imperio et al. 2012).

A critical reading of this literature puts into light several methodological shortcomings: i) multicollinearity among explanatory variables (Richard et al. 2010; Ferretti et al. 2012) (ii) erroneous handling of non-normal and non-continuous distributions of the response variable (Imperio et al. 2012), and (iii) problems in inferring cause-effect relationships, so that no firm decision could be established between climatic and environmental influence or inter-intra specific competition affecting growth rate of ungulate population.

Multicollinearity, which occurs when two or more predictors in a multiple regression model are highly correlated, leads to variance inflation and increase type-I errors, thus making some of the coefficients appear significant when they are not (Zuur et al. 2010).

Another important source of bias depends on erroneous handling of non-normal and non-continuous distributions of the response variable. In many cases, this problem is dealt with using square root or logarithm transformations (Imperio et al. 2012) but despite this procedure is recommended in general biometry textbooks (e.g., Sokal & Rohlf 1995), its validity is restricted to cases when deviations from normality are not too excessive. Moreover, discrete response variables containing many zeros cannot be transformed into normal distributions, and inference is doomed to be severely biased (O'Hara & Kotze 2010; Zuur et al. 2012; Lombardi et al. 2017).

There are concerns related to the link between correlations and causation, which are tricky to deal with. Endogenous variables may, in fact, appear unrelated when they are related, or on the contrary, they may be correlated even when no causal link is present. A spurious or missing correlation may arise for several reasons which include (i) a common causation that induces a false relationship or cancels out an existing association, (ii) a reciprocal association loop, (iii) a conditional relationship between explanatory and response variables following the value of a third control variable, or (iv) a non-linear association between dependent and independent variables (Shipley 2000; Navidi 2006; McDonald, 2014; Kendall, 2015). When a correlation between two variables is detected, cause-effect relationships cannot be easily deduced without further assumptions (Shipley 1999; Shipley 2000). The best way to test causal relationships is to use a proper experimental design where the hypothetical cause is directly manipulated (Shipley, 1999). However, manipulative experiments are difficult to achieve, and researchers have to rely mainly on observational studies.

The problem of inferring cause-effect relationships among variables can be addressed by path analysis or Structural Equation Models (SEM) (Pearl et al. 2016). In field studies often the variables of interest cannot be directly recorded by the observers. For instance, we cannot measure the "habitat quality" or the "climate". However, we can measure some caracteristic we expect to be correlated to "habitat quality" or the "climate" and so obtain an indirect evaluation of the variable of interest. This is the same done in principal component analysis: a reduced number of meaningful factors are estimated from the correlations among a large number of descriptors. In SEM terminology, we refer to the unobservable factors as latent and to the observed descriptors as manifest. A SEM is a combination of a measurement model that defines latent variables using one or more manifest variables and a structural model that imputes causal relationships between latent variables

(Shipley, 2000). The development of a measurement model is also important to control for the errors introduced during observations, i.e., it represents a state space model for the unobserved variables of interest. In this way, a latent variable is not directly observed, but its existence is inferred by the way it influences manifest variables that can be directly observed (Shipley, 2000).

One known limitation of standard SEM is to assume that all variables are normally distributed (Grace et al. 2012). The introduction of Generalized Structural Equations Models (GSEM), may overcome this limitation. In GSEM, it is possible to have a model with both continuous and discrete variables grouped together in the same construct. As such, GSEM combines the power and flexibility of both SEM and GLM in a unified modeling framework. The advantages of GSEM are: (i) to evaluate potential causal relationships with the "structural model"; (ii) to consider both direct and indirect effects of multiple interacting factors, simultaneously (Shipley 2000; Pearl et al. 2016; Agresti 1990; Muthén & Asparouhov 2015); (iii) the possibility of using appropriate probability density functions other than the normal one for manifest indicators and latent constructs.

In this paper we propose a novel approach based on Structural Equation Model (SEMs) and Generalized Structural Equations Modelling (GSEMs). In particular, we tested for the effects of climatic and environmental factors, of the density of potential competitors and density dependence on the growth rate of wild boar, fallow and roe deer for the whole study period. The main hypothesis is that density dependence and competition are more relevant than climatic and environmental factors in regulating Mediterranean ungulate populations, in contrast with ungulates living in harsher and more fluctuating environments. The predictive performances of the different methods were compared using information theoretic indexes (AIC and BIC), and precision of regression coefficients (CV).

Materials and Methods

Study area and data collection

Our study took place in the Preserve of Castelporziano near Rome (Italy), is an area covering 48 km^2. The habitat is characterized by an old-growth natural oak wood, with both evergreen *(Quercus ilex* and *Q. suber)* and deciduous (mainly *Q. cerris* and *Q. frainetto*) tree species. A detailed description of the vegetation of the study area can be found in Bianco et al. 2001. Information on ungulate populations are given in Focardi et al. (2015) and Imperio et al. 2012. The details of Preserve history are described in Imperio et al. (2010). No large predators were present in the Preserve through the study and poaching was prevented thanks to careful surveillance by gamekeepers. The dataset used to estimate the meta-model for the community of ungulates in the Preserve of Castelporziano (Italy) in the period 1878–1986 was represented by climatic, enviromental, population density and population growth rate.

Climate

We used the meteorological data recorded by the station of the Collegio Romano in the city of Rome at about 15 km from the Preserve to determine climatological conditions (Imperio et al. 2012). Monthly precipitation and mean temperature data are available for the whole study. From meteorological data, we computed the Gaussen index (GI, Dajoz 2006) for early spring (March–April, GI_{ma}), late spring (May–June, GI_{mj}), and summer (July–August, GI_{ja}), as the total amount of precipitation (in mm) minus twice the mean temperature (in °C). The Gaussen index is a measure of the water available for vegetation, and can therefore be used as a proxy for resource availability (Toïgo et al. 2006). Wet conditions correspond to large GI values, while dry and/or drought conditions correspond to low values. The value of GI during the summer provides information on the severity of summer drought, and thus, indirectly, on the quality and quantity of food available during the harshest season for this type of environment.

Habitat quality

Castelporziano Preserve includes one of the most important relict Italian Mediterranean forests (Pignatti et al. 2001). At present, a small percentage of land is used for cultivations and pastures. Natural woods were usually exploited until about three decades ago; clear-cuttings were practiced only in case of need of soil tilling, in particular during the 1940s. Later, most of these lands were reforested, mainly with domestic pine *Pinus pinea* or cork oak *Q. suber*. Reconstruction of past land cover of the Preserve was made possible by the availability of a cadastral map (dated 1867) and of a set of aerial photographs taken in 1930, 1954, 1959, 1969 and 1980 (source: Istituto Geografico Militare, Florence, Italy) and 1943 (source: Royal Air Force, Pigorelli Museum, Rome, Italy) (Imperio et al. 2010). The main types of vegetation cover determined from these data are *natural woods (NW)*, open areas and planted stands, *pine plantation (PP)* (mainly domestic pine).

Population density

Population density estimates are obtained from the data base of bag records described by Imperio et al. (2010). Data are available for the period 1878 to 1986. Hunting was carried out every year, from November to March of the following year. Density estimates (Nt) were computed as the number of specimens killed during hunting drives, per km^2 of driven area. The quality of collected data and their validity for the assessment of population density are discussed by Imperio et al. (2010), which showed no relevant bias due to hunting. We used wild boar density (*WBD*), roe deer density (*RDD*) and fallow deer density (*FDD*).

Population growth rate

We applied a deterministic model for a population with discrete breeding periods. We used a discrete exponential population growth model in wich the discrete growth rate is defined as:

$$ln[N_t] = ln[N_0] + rt$$

where $[N_t]$ is the population size at time t (years), and N_0 is the initial population size. In presence of intra-specific competition for limited resources, or of density-dependent effects (e.g. greatly depressed by human activities or disease transmission) the growth rate depends on population density. Two of the simplest models for density dependence are the stochastic Gompertz (1825), and Ricker models (1979) (a review of Tjørve & Tjørve, 2017). Using density dependence models (Gompertz and Ricker models) we calculate roe deer growth rate (*RDGr*), fallow deer growth rate (*FDGr*) and wild boar growth rate (*WBGr*).

Hunting effort

Actual harvest rate (*HR*, number of animals killed/ number of animal counted) is available for a limited number of years (1906–1942), for which direct counts of all the species are available. As a proxy for HR, we used a measure of *Hunting effort* (*HE*). The definition of *Hunting effort* is the total number of shooting days per hunting season. Shooting plans (based on animal counts) were in fact put into practice by deciding the number of hunting drives to be carried out. Further details on study area, data collection, are provided in S1 Fig 1 in Chapter 1 Supporting information. Data validation and measures computations are provided in Imperio et al. 2010 and Imperio et al. 2012.

Structural equation modelling

A SEM requires the *a-priori* definition of links among model variables in the form of a regression equations system. The goal of this class of models is to minimize the difference between estimates and expectations variance-covariance matrix of data.

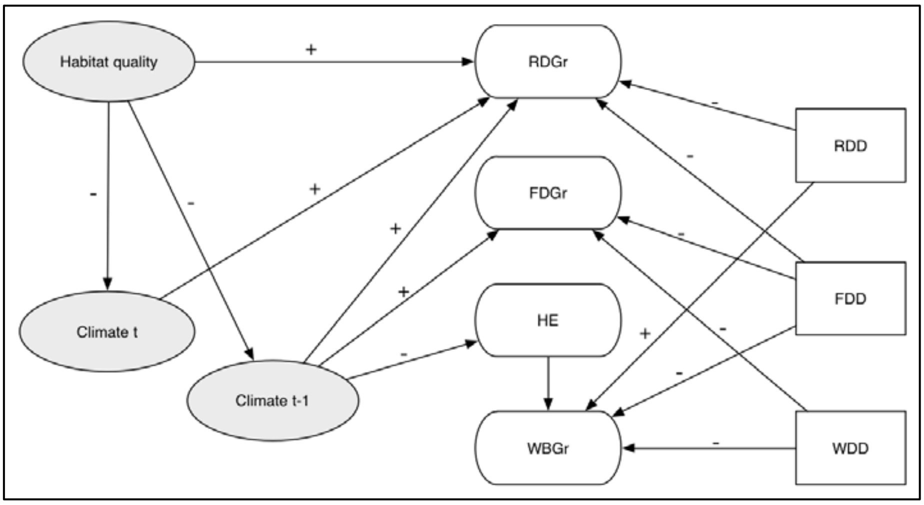
Latent variables are unobserved factors denoted, η_1, η_2,..., η_n, that represent an hypothetical construct that can be inferred by the way it influences manifest or observed variables (continuous, $Y_i=y_1, y_2,..,y_n$), (Shipley, 2000; Muthén & Asparouhov , 2015).

A SEM model is composed by two sub-models: a measurement model that describes the relationships between latent variables and their manifest variables and a structural or causal model that constitutes a directional chain system that describes the hypothetical causal relationship between the constructs of theoretical interest (latent variables) using *path diagrams* (Fig 1).

Structural coefficients or regression coefficient (γ, β, λ) represent the effects of each independent variable on the dependent variable (Fig 1).

A manifest variable, in a SEM with latent variables, plays a role of endogenous variable if it is predicted by another variable in the model and is therefore a response variable; it is assumed to be generated as a linear function of its latent dimension and the residual error term represents the imprecision in the measurement process.

Figure 1 Path diagrams. Initial meta-model representing the hypothesized causal relationships.



Variable names are: Habitat quality = measured by the main types of vegetation, NW and PP; Climatet = measured by Gaussen Index that represent climatic condition in current year; Climatet-1 = measured by Gaussen Index that represent climatic condition in previous year; RDGr= Roe deer growth rate stimated with the stochastic Gompertz (1825), and Ricker models (1979); FDGr = Fallow deer growth rate; WBGr = Wild boar growth rate; RDD= Roe deer density were computed as the number of specimens killed during hunting drives, per km2 of driven area; FDD=Fallow deer density; WBD= Wild boar density; HE= Hunting effort measured as the total number of shooting days per hunting season .The number of observations is the same for all models (N=104). Symbols and variables are described in the text and in Table 1. In grey are showed the latent variables.

Generalized Structural Equation Models

GSEMs represent a generalization of SEMs by allowing the use of discrete variables and non-Gaussian distributions. They combine observed (or manifest) and latent variables representing unmeasured constructs. A GSEM (Bollen & Pearl 2013) reads:

$$\begin{aligned} \boldsymbol{\eta} &= f_{\eta}(\eta, \xi, \zeta) \\ \mathbf{x} &= f_{x}(\eta, \delta) \qquad \text{(eqns 2)} \\ \mathbf{y} &= f_{y}(\eta, \varepsilon) \end{aligned}$$

where x and y are vectors of manifest variables and η, ξ, ζ represent the latent variables, while δ, and ε denote the error terms. The functions (f_{η}, f_{y}, f_{x}) provide a general way to represent the connections between the variables within the parentheses to those on the left hand side of each equation.

We developed a causal model, assuming that *HE* is influenced by climatic condition and on the other hand is a mediator variable between climatic condition of previous year and wild boar density.

We verified that the number of parameters is identifiable according to Shipley (Shipley, 2000). We used a robust maximum likelihood estimator and a sandwich estimator (Cameron & Trivedi, 2009). We fitted GSEMs with Mplus (Muthén & Muthén, 2015). The Mplus codes used to generate SEM and GSEM models are presented in S1 Appendix.

Statistical analysis

We compared two modelling approaches described in Lombardi et al (2017). We decided to use them due to their widespread use in the pertinent literature on ecology (Imperio et al. 2012; Grace et al 2016). In he tested models *HE* is a mediator variable. Note that *HE* is discrete variable by definition (because it is a count) and hence cannot be assumed to be normally distributed.

Following the approaches described in the literature, we first used Structural Equation Models with the response variable *HE* erroneously untransformed (normally distributed). In Fig 2 we show *HE* distribution and log-transformed HE. We test the distribution and we assuming that *HE* follows a Zero Inflated Negative Binomial distribution (ZINB).

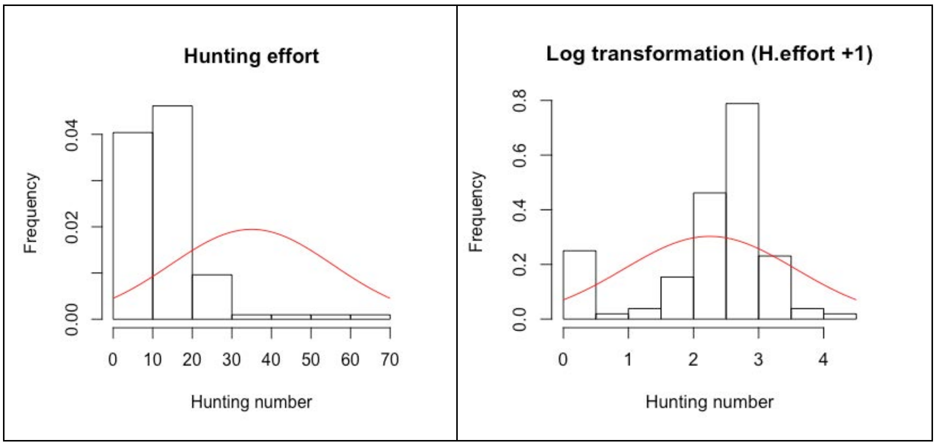
In the ZINB models a logit model governs the binary outcome of whether a count variable has a zero or positive realization. When the realization is positive the conditional distribution is modelled by a negative binomial at zero count data model.

For SEM and GSEM we considered all significant ($P<0.05$) and non-significant coefficients.

Akaike information criterion (AIC) and Bayesian information criterion (BIC) were also computed to assess model performances ((Akaike index: Akaike, 1974; Bayesian information criterion: Stone, 1979).

Statistical analysis was carried out in R (R Core Team, 2016) using the packages fitdistrplus, gamlss.

Fig 2. Hunting effort distribution before (left panel) and after log-transformation (right panel).



The continuous red line shows the theoretical normal curve for reference

Models' comparison

We compared models by two different approaches. First, we compared SEM and GSEM with AIC and BIC. Second we performed an analysis of models and we measured the precision of each estimated regression coefficient $\widehat{\beta}$ by computing its coefficient of variation ($CV = \frac{SE(\widehat{\beta})}{|\widehat{\beta}|} = \frac{1}{|\widehat{\beta}|/SE(\widehat{\beta})} = \frac{1}{|T|} = \sqrt{\chi_1^2}$, where T is the statistic test and χ_1^2 is the chi-square test with one degree of freedom). For a more general evaluation of the model's precision, we computed the median CV for the parameters estimated by each model (Lande, 1977).

Definition of working hypotheses

In this paper, we follow the Grace (2007) approach that stresses the usefulness of SEM in wildlife studies and for ecological complex network (Grace et al. 2016). It represents a framework for evaluating and comparing complex hypotheses developed from a theoretical knowledge about the processes responsible for data structure. SEMs and GSEMs are useful because it is possible to deduce latent variables (i.e. variables which cannot be directly estimated in the field) from manifest, measured, correlated variables (similarly to what ecologists do when using the factorial analysis). Relevant, using SEM and GSEM, it is possible to test causal relationships, accounting for spurious correlations among variables. First, we developed a theoretical model of the studied guild of ungulates. This was made on the basis of preliminary results using GLM approach in Imperio et al. 2012. We performed a confirmatory approach, to which extent data matched our theory. We used SEM and GSEM to develop a general model of the community of ungulates through the whole study period. We have assumed, according to literature, the existence of three latent variables: ξ_1 represents climatic condition of current year and is described by GI_{mat}, GI_{mjt} *and* GI_{jat} ; ξ_2 represents climatic condition of previous year and is described by GI_{mat-1}, GI_{mjt-1} *and* GI_{jat-1}; η_1 represents the habitat quality (*Natural Wood* and *Pine Plantation*). In the model η_1 is measured by ξ_1 and ξ_2. The use of latent variables allowed us to reduce the unavoidable errors in the measurement of manifest variables.

The structure of the models corresponding to measurement part and structural part is shown in Fig 1. We test that η_1 is measured by ξ_1 and ξ_2 or in other words the habitat quality depends on climatic condition respectively of current and previous year. We assume that climate variables and habitat quality influence growth rates of roe deer (*RDGr*) and fallow deer (*FDGr*). We test a causal link between climate of the previous year and *HE* and we related *HE* to *wild boar density (WBD)*. We assume that *roe deer growth rate (RDGr)* depending on *roe deer* (*RDD*) and *fallow deer density (FDD)*, *fallow deer growth rate (FDGr)* depending on *fallow deer (FDD)* and *wild boar density (WBD)*, finally *wild boar growth rate (WBGr)* is caused by *wild boar (WBD)*, *fallow deer (FDD)* and *roe deer density (RDD)*.

Note that SEM and GSEM allows us to study the effects of remote and proximate causes of population dinamics of three ungulate species in the same statistical framework. Further, the use of latent variables reduces the unavoidable errors in the measurement of manifest variables. Once the measurement model is defined, we can establish appropriate causal relationships among latent variables according to literature.

The mesurement part is implemented by the following system of regression equations (Fig 1):

$$NW = \lambda_1 \eta_1 + \delta_{NW}$$

$$PP = \lambda_2 \eta_1 + \delta_{PP}$$

$$GI_{mat} = \lambda_3 \xi_1 + \varepsilon_{GImat}$$

$$GI_{mjt} = \lambda_4 \xi_1 + \varepsilon_{GImjt} \qquad \text{(eqns 2)}$$

$$GI_{jat} = \lambda_5 \xi_1 + \varepsilon_{GIjat}$$

$$GI_{mat-1} = \lambda_6 \xi_2 + \theta_{GImat-1}$$

$$GI_{mjt-1} = \lambda_7 \xi_2 + \theta_{GImjt-1}$$

$$GI_{jat-1} = \lambda_8 \xi_2 + \theta_{GIjat-1}$$

The structural or causal part is implemented by the following system of regression equations (Fig 1):

$$RDGr = \lambda_9 RDD + \lambda_{10} FDD + \lambda_{11} \eta_1 + \lambda_{12} \xi_2 + \lambda_{13} \xi_1 + \delta_{RDGr}$$

$$FDGr = \lambda_{14} FDD + \lambda_{15} WBD + \lambda_{16} \xi_2 + \delta_{FDGr} \qquad (eqns3)$$

$$WBGr = \lambda_{17} FDD + \lambda_{18} RDD + \lambda_{19} WBD + \lambda_{20} HE + \delta_{WBGr}$$

$$HE \sim ZINB(\mu, v),\ \log(\mu\ (HE)) = \lambda_{21} \xi_2 .$$

Table 1. Variables and symbols used in SEM and GSEM.

Variable name	***Definition***
Habitat quality (η_1)	Latent variable measured by *natural wood* and *pine plantation*
Natural wood (NW)	The main types of vegetation cover determined by open areas and planted stands
Pine plantation (PP)	The main types of vegetation cover determined by domestic pine plantation
Climate$_t$ (ξ_1)	Latent variable measured by Gaussen Index that represent climatic condition **in current year**
GI_{mat}	Gaussen Index for early spring (March–April) as the total amount of precipitation (in mm) minus twice the mean temperature (in ° C)
GI_{mjt}	Gaussen Index late spring (May–June)
GI_{jat}	Gaussen Index and summer (July–August)
Climate $_{t-1}$(ξ_2)	Latent variable measured by Gaussen Index that represent climatic condition **in previous year**
GI_{mat-1}	Gaussen Index for early spring (March–April) as the total amount of precipitation (in mm) minus twice the mean temperature (in ° C)
GI_{mjt-1}	Gaussen Index late spring (May–June)
GI_{jat-1}	Gaussen Index and summer (July–August)
Roe deer (RDGr)	*Roe deer growth rate* stimated with the stochastic Gompertz (1825), and Ricker models (1979)

Variable name	***Definition***
Roe deer density (RDD)	*Roe deer density* were computed as the number of specimens killed during hunting drives, per km^2 of driven area
Fallow deer (FDGr)	*Fallow deer growth rate*
Fallow deer density (FDD)	*Fallow deer density*
Wild boar (WBGr)	*Wild boar growth rate*
Wild boar density (WBD)	*Wild boar density*
Hunting effort (HE)	*Hunting effort* measured as the total number of shooting days per hunting season

RESULTS

The distribution of *HE* is showed in Fig 2. In most of the years (12.5%) we had no *hunting*. The number of *HE* ranged from 0 to 66, and the distribution has high kurtosis (3.72) and skewness (-1.20).

The distribution of *HE* is best fitted by a Zero Inflated Negative Binomial distribution, which is much better supported than alternative models (NB, DAIC = 18.68, ZIP, DAIC =211.9; Poisson, DAIC=487.86). Data transformation changes the discrete *HE* distribution into a continuous one, which remains, however, non-normally distributed (Shapiro-Wilk Test: log(*H. effort* +1), *W*=0.829, P<0.001) (Fig.2).

Structural Equation Models

The path diagram of the estimated model is reproduced in Fig. 1, and the parameter estimates are reported in Table 2 for SEM and Table 3 for GSEM. The variance-covariance/correlation matrix used in SEM and GSEM is shown in S1 Table. The measurement part of the model (Fig 1) allow to evaluate three latent variables, namely, habitat quality, climate of year t and climate of year t-1. According to Imperio et al (2012) we found that climatic variables were well represented by the respective Gaussen indexes computed for March–April, May–June and July–August for previous year. We confirm that the Gaussen index for July–August was less significant than in other periods. Habitat quality is a construct that depends on both habitat composition and the amount of rainfall. Thus the effect of climate could be both direct and indirect, through modification of plant productivity. Following Imperio et al. (2012) we have introduced paths between climate variables and habitat quality only for the growth rates of roe and fallow deer, but these were not significant. We have introduced a link between climate of the previous year and hunting effort and the second was strongly related to climate (λ_{21} = 0.476 (0.075), p = <0.001). Intra-specific competition was likely to have an important role for all three species, and in addition the effect of inter-specific competition is well represented by coefficients $\lambda_{10}, \lambda_{17}, \lambda_{18}$.

To select the appropriate distribution of *HE* for GSEM, we first selected the discrete distributions available in Mplus. It resulted that four of discrete distributions, Poisson, ZIP, and Negative binomial and ZINB were supported. We tested the distributions, and we selected the Zero Inflated Negative Binomial distribution for *HE*.

If we implement the SEM and GSEM models with Mplus, they converge without warning message. The fit of these models was satisfactory as shown by the small CV values, particularly those obtained from GSEM model. Indeed even GSEM provided a better fit than SEM (ΔAIC =4289-4214=77, ΔBIC =4261-4186=75). In synthesis, this analysis shows that the GSEM model is always better than SEM, since it has lower AIC and BIC. Path coefficients and CV values for SEM and GSEM models are shown in Table 2 and Table 3.

Model comparisons

The comparison of the SEM and GSEM models is reported in Table 2-3. It clearly appears that the precision of GSEM models is higher than that of the corresponding SEM models. Considering the median CV values, SEM is less precise (median CV = 0.687) than GSEM (median CV = 0.528), whose coefficient CV values range from 0.14 to 4.88.

Interestingly the number of regression coefficients that are significant is maximal in GSEM with respect to SEM (Table 2-3). Since the results indicate that GSEM is the most appropriate model for our data set (lower AIC/BIC, and lower CV median), it is interesting to investigate selected non-standardized partial effect sizes (and standard errors) ranked by magnitude and propose interpretations for this model (Table 4). The path diagram of Fig. 2 illustrates how both intra- and interspecific competitions had a stronger effect than habitat quality (η_1) and climate (ξ_1, ξ_2) on the growth rates of the three studied species. Although these populations were mainly regulated by density dependence, also inter-specific relationships appeared to play an important role, especially for roe deer, which suffered fallow deer competition (λ_{10}).

There were reciprocal negative path coefficients between fallow deer density and wild boar growth rate which appeared to be stronger on wild boar (λ_{17}) then on fallow deer (λ_{15}). A weak facilitation effect of roe deer on wild boar was found instead (λ_{18}). Climate conditions of previous year (ξ_2) weakly influenced negatively the *hunting effort* (λ_{21}) which in turn was most strongly related to wild boar density (λ_{20}).

Table 2. Non-Standardized path coefficients, SE, coefficient of variation (CV) of regression parameters and their median and p-value in SEM.

Variables	Path coefficients	SEM		
		Estimate ± SE	CV	P
Habitat quality (η_1)				
Natural wood	λ_1	1.211 ± 0.222	0.183	**<0.001**
Pine plantation	λ_2	-1.739 ± 0.267	0.153	**<0.001**
Climate$_t$ (ξ_1)				
GI_{mat}	λ_3	0.173 ± 0.125	0.722	0.167
GI_{mjt}	λ_4	0.128 ± 0.081	0.633	0.112
GI_{jat}	λ_5	-0.035 ± 0.051	1.457	0.493
Climate $_{t-1}$(ξ_2)				
GI_{mat-1}	λ_6	0.152 ± 0.113	0.743	0.178
GI_{mjt-1}	λ_7	0.111 ± 0.074	0.667	0.131
GI_{jat-1}	λ_8	-0.028 ± 0.038	1.357	0.454
R. deer by R. deer density	λ_9	0.111 ± 0.046	0.414	**0.015**
R. deer by F. deer density	λ_{10}	-0.070± 0.038	0.543	**0.064***
η_1 on R. deer	λ_{11}	-0.179 ± 0.697	3.893	0.797
ξ_1 on R. deer	λ_{12}	0.148 ± 0.192	1.297	0.440
ξ_2 on R. deer	λ_{13}	0.039 ± 0.305	7.820	0.899

F. deer by F. deer density	λ_{14}	-0.126 ± 0.059	0.468	**0.032**
F. deer by W. boar density	λ_{15}	-0.096 ± 0.066	0.687	0.143
ξ_2 on F. deer	λ_{16}	0.046 ± 0.090	1.956	0.606
W. boar by F. deer density	λ_{17}	-0.118 ± 0.039	0.330	**0.002**
W. boar by R. deer density	λ_{18}	0.064 ± 0.030	0.469	**0.033**
W. boar by W. boar density	λ_{19}	-0.195 ± 0.079	0.405	**0.013**
H. effort on W. boar density	λ_{20}	-0.071 ± 0.014	0.197	**<0.001**
ξ_2 on H. effort	λ_{21}	-1.070 ± 0.894	0.835	0.231
η_1 by ξ_1	γ_1	1.303 ± 1.026	0.787	0.204
η_1 by ξ_2	γ_2	1.614 ± 1.222	0.757	0.186

Variables and symbols are detailed in Table1. The number of observations is N=104.

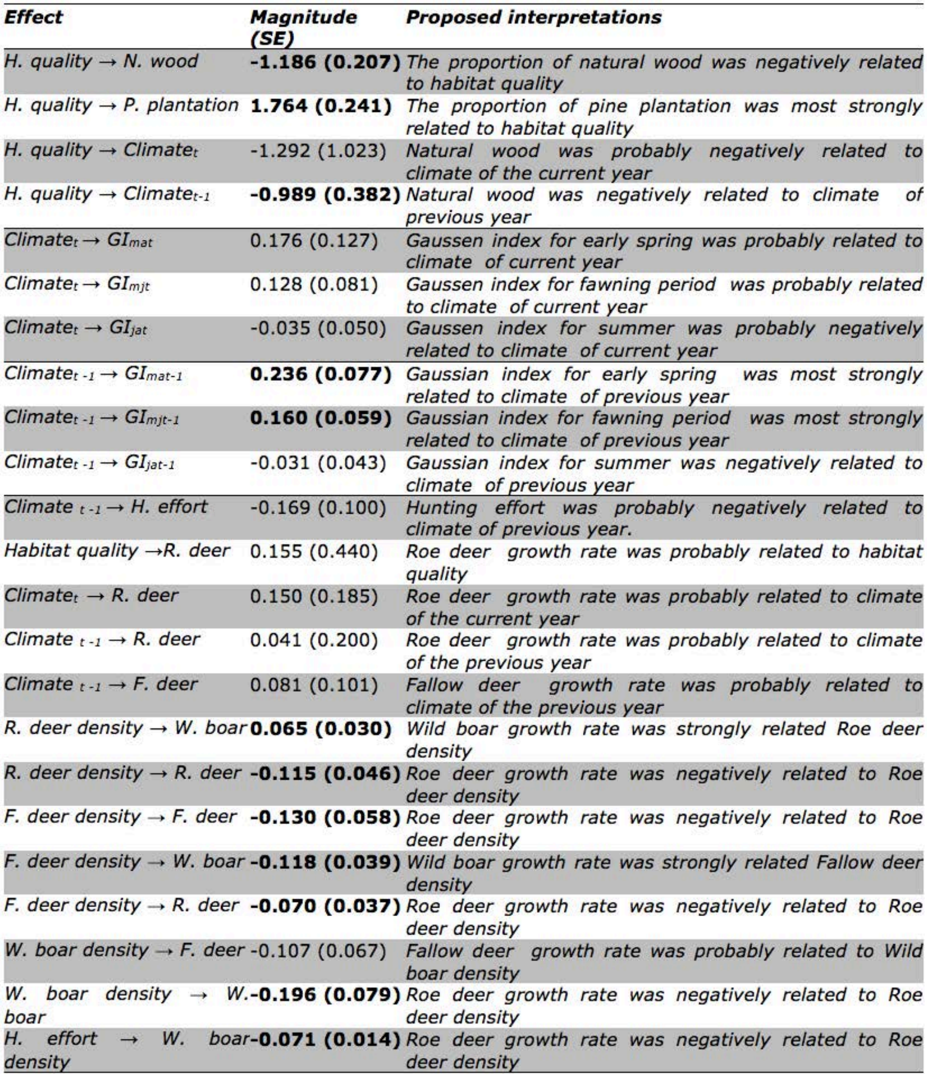
Table 3. Non-Standardized path coefficients, SE, coefficient of variation (CV) of regression parameters and their median and p-value in GSEM.

Variables	**Path coefficients**	**SEM**		
		Estimate ± SE	**CV**	**P**
Habitat quality (η_1)				
Natural wood	λ_1	-1.186 ± 0.207	0.174	**<0.001**
Pine plantation	λ_2	1.764 ± 0.241	0.136	**<0.001**
Climate$_t$ (ξ_1)				
GI_{mat}	λ_3	0.176 ± 0.127	0.721	0.166
GI_{mjt}	λ_4	0.128 ± 0.081	0.633	0.112
GI_{jat}	λ_5	-0.035 ± 0.050	1.428	0.489
Climate $_{t\ -1}$(ξ_2)				

GI_{mat-1}	λ_6	0.236 ± 0.077	0.326	**0.002**
GI_{mjt-1}	λ_7	0.160 ± 0.059	0.369	**0.007**
GI_{jat-1}	λ_8	-0.031 ± 0.043	1.387	0.474
R. deer by R. deer density	λ_9	-0.115 ± 0.046	0.400	**0.012**
R. deer by F. deer density	λ_{10}	-0.070± 0.037	0.528	**0.060**[*]
η_1 *on R. deer*	λ_{11}	0.155± 0.440	2.838	0.726
ξ_1 *on R. deer*	λ_{12}	0.150± 0.185	1.233	0.416
ξ_2 *on R. deer*	λ_{13}	0.041± 0.200	4.878	0.836
F. deer by F. deer density	λ_{14}	-0.130 ± 0.058	0.446	**0.025**
F. deer by W. boar density	λ_{15}	-0.107 ± 0.067	0.626	0.144
ξ_2 *on F. deer*	λ_{16}	0.081 ± 0.101	1.247	0.427
W. boar by F. deer density	λ_{17}	-0.118± 0.039	0.330	**0.002**
W. boar by R. deer density	λ_{18}	0.065± 0.030	0.461	**0.033**
W. boar by W. boar density	λ_{19}	-0.196± 0.079	0.405	**0.013**
H. effort on W. boar density	λ_{20}	-0.071± 0.014	0.197	**<0.001**
ξ_2 *on H. effort*	λ_{21}	-0.169± 0.100	0.591	0.089
η_1 *by* ξ_1	γ_1	-1.292 ± 1.023	0.792	0.207
η_1 *by* ξ_2	γ_2	-0.989 ± 0.382	0.386	**0.010**

Variables and symbols are detailed in Table1. The number of observations is N=104.

Table 4. Select non-standardized partial effect sizes (and standard errors) in GSEM ranked by magnitude and proposed interpretations.



Effect	***Magnitude (SE)***	***Proposed interpretations***
H. quality → N. wood	**-1.186 (0.207)**	*The proportion of natural wood was negatively related to habitat quality*
H. quality → P. plantation	**1.764 (0.241)**	*The proportion of pine plantation was most strongly related to habitat quality*
H. quality → Climate$_t$	-1.292 (1.023)	*Natural wood was probably negatively related to climate of the current year*
H. quality → Climate$_{t-1}$	**-0.989 (0.382)**	*Natural wood was negatively related to climate of previous year*
Climate$_t$ *→ GI*$_{mat}$	0.176 (0.127)	*Gaussen index for early spring was probably related to climate of current year*
Climate$_t$ *→ GI*$_{mjt}$	0.128 (0.081)	*Gaussen index for fawning period was probably related to climate of current year*
Climate$_t$ *→ GI*$_{jat}$	-0.035 (0.050)	*Gaussen index for summer was probably negatively related to climate of current year*
Climate$_{t-1}$ *→ GI*$_{mat-1}$	**0.236 (0.077)**	*Gaussian index for early spring was most strongly related to climate of previous year*
Climate$_{t-1}$ *→ GI*$_{mjt-1}$	**0.160 (0.059)**	*Gaussian index for fawning period was most strongly related to climate of previous year*
Climate$_{t-1}$ *→ GI*$_{jat-1}$	-0.031 (0.043)	*Gaussian index for summer was negatively related to climate of previous year*
Climate $_{t-1}$ *→ H. effort*	-0.169 (0.100)	*Hunting effort was probably negatively related to climate of previous year.*
Habitat quality →R. deer	0.155 (0.440)	*Roe deer growth rate was probably related to habitat quality*
Climate$_t$ *→ R. deer*	0.150 (0.185)	*Roe deer growth rate was probably related to climate of the current year*
Climate $_{t-1}$ *→ R. deer*	0.041 (0.200)	*Roe deer growth rate was probably related to climate of the previous year*
Climate $_{t-1}$ *→ F. deer*	0.081 (0.101)	*Fallow deer growth rate was probably related to climate of the previous year*
R. deer density → W. boar	**0.065 (0.030)**	*Wild boar growth rate was strongly related Roe deer density*
R. deer density → R. deer	**-0.115 (0.046)**	*Roe deer growth rate was negatively related to Roe deer density*
F. deer density → F. deer	**-0.130 (0.058)**	*Roe deer growth rate was negatively related to Roe deer density*
F. deer density → W. boar	**-0.118 (0.039)**	*Wild boar growth rate was strongly related Fallow deer density*
F. deer density → R. deer	**-0.070 (0.037)**	*Roe deer growth rate was negatively related to Roe deer density*
W. boar density → F. deer	-0.107 (0.067)	*Fallow deer growth rate was probably related to Wild boar density*
W. boar density → W. boar	**-0.196 (0.079)**	*Roe deer growth rate was negatively related to Roe deer density*
H. effort → W. boar density	**-0.071 (0.014)**	*Roe deer growth rate was negatively related to Roe deer density*

Discussion

This paper presented a long-term analysis of the population dynamics of a community of ungulates living in a Mediterranean climate and integrated the research presented by Imperio et al. (2012). Data validation (Imperio et al. 2010) indicated that the effects described in this paper were not due to the hunting regime carried out in the Preserve. We investigated the role of intra- and inter-specific competition on the dynamics of three ungulate species, together with the effects of both environmental and climatic factors. The main hypotheses tested in this work were that 1) the complexity of the ungulate community influences intra- and inter-specific interactions; and 2) in Mediterranean ungulate communities, exposed to relatively mild climate fluctuations, trophic interactions such as density dependence, competition and facilitation have stronger effects than environmental controls such as climate (explored by a SEM and GSEM approach). The results of all analyses confirmed that intra-specific competition was a main ingredient in the regulation of the growth rates within the ungulate community at Castelporziano, in keeping with the view that density-independent regulation is more important in those populations facing strong climatic fluctuations (Post 2005). Inter-specific competition appeared to be generally of lower importance, although evidences of interactions among species were found. Perhaps the most relevant impact was that of fallow deer on the dynamics of roe deer, presumably as a consequence of the very high fallow deer density at that time. Results of the GSEM model suggest that the observed inverse correlation between fallow and roe deer densities was not a side effect of spurious correlations with unknown variables but, instead, was due to a direct competition between the two species (Putman 1996). This finding is consistent with the available information on inter-specific competition in Mediterranean environments (Focardi et al. 2006, Ferretti et al. 2008). Other effects included the negative influence of wild boar on fallow deer, the negative effect of red deer on wild boar, and the apparent positive effect of roe deer on red deer and wild boar. The interpretation of these latter effects is however less straightforward. Results of this study suggest that the negative effect of wild boar on fallow deer was probably indirect, related to the impact of selection hunting to control wild boar. The higher the wild boar density, the more intense presumably was the hunting pressure, with a larger number of hunting drives affecting also other ungulates such as fallow deer. On the contrary, the apparent facilitation exerted by roe deer could be caused by a spurious correlation with a third unknown variable. A pattern of positive correlation between wild boar and roe deer was also observed by Mysterud et al. (2007) in Poland, but it appears theoretically difficult to imagine which mechanisms were operating. A possible explanation is that both species were positively influenced by acorn production: since the two species are polytocous, they could be in the best biological conditions to exploit the window of opportunity represented by masting.

The hunting effort seems to play an important role in determining the growth rate of wild boar as opposed to what was found by Imperio et al. (2012) in which they did not find any association between HE and any of these species, except for fallow deer in the post-removal period.

Habitat composition and climate have been shown to affect to some degree the three species. However, these effects had a secondary importance with respect to intra- and interspecific competition in regulating the ungulate populations studied here. Habitat factors entered the models in terms of latent variable 'habitat quality' in the SEM and GSEM model. Unfortunately, no information on actual resource availability (e.g. mast production) was available. As for climate, very wet conditions in May–June appeared to play a negative role for all deer species, probably being associated with insurgence of thermoregulatory problems in fawns (Van Moorter et al. 2009). On the contrary, water availability during the spring of the previous year had a positive effect on both fallow and roe deer. This effect is presumably due to increased primary productivity during moist springs, which in turn improves body conditions in young females and thus enhances their fecundity (Gaillard et al. 1992). In fact reproductive success of roe deer depends mainly on environmental conditions, in spring-summer (McLoughlin et al. 2010; McLoughlin et al. 2007; Pettorelli et al. 2005). Neither of these factors, however, had any effect on wild boar dynamics. This result is consistent with observations on modern data, showing a weak effect of climate on this species and mainly on subadult males (Focardi et al. 2008). Finally, summer drought has been shown to play an almost insignificant role in the dynamics of the studied species. In general the summer Gaussen index values fluctuated widely during the entire span of the study period but no clear downward trend was observed. However, our data set ends in 1986, whereas the mean annual temperature started to grow more rapidly afterwards (Brunetti et al. 2006). The importance of climatic factors, and in particular of summer droughts, could thus grow in coming years, as climate change projections indicate an expected increase of aridity and of heat waves in most Mediterranean regions (Gao & Giorgi 2008). The results of this study provided also important information on the population dynamics of individual species. Fallow deer appeared not to be much influenced by the other herbivores. This independence is presumably linked to its trophic position (between intermediate and roughage eaters) in the community of ungulates (Hofmann 1989), as fallow deer is able to exploit low quality browse but also to benefit from the flushing of grasses during some periods of the year. For this species, the final regulatory mechanism was represented by a combination of climatic factors and density dependence, together with a positive effect of the amount of pine plantations.

Direct density-dependence explained the growth rate of roe deer at a much lower extent, being influenced by habitat factors (in particular by a negative impact of pine woods), climate and competition with fallow deer. These observations are consistent with life history theory of ungulates. Density dependence in roe deer is well established even in south-European populations (Focardi et al. 2002; Kjellander et al. 2006), but in previous study cases roe deer had no competitors. At Castelporziano, density dependence was operating but it was accompanied by other relevant regulatory processes.

The wild boar population appeared to be scarcely linked to the other ungulates. Theoretical considerations (Focardi et al. 2008) suggest in fact that the demography of this suid is quite different from that of the other species.

This study was made possible by the availability of an exceptionally long data set: because of the peculiar management model of Castelporziano, bag records and other types of information were carefully conserved for more than one century. This allowed for estimating harvest density (Imperio et al. 2010); in addition, climate records and the availability of unusually old aerial photographs allowed to carefully track habitat variations in the area. We have to note that an important drawback of the data analyzed here was the lack of information on population structure. In addition, the data were characterized by a high level of noise and by several years during which population sampling was poor, a problem that was addressed by the use of a SSM to filter the data (Imperio et al. 2012) prior to the SEM and GSEM analyses. The results presented here should be used to formulate working hypotheses to be verified with recent data, collected in a more controlled way, in order to disentangle the role of competition and climatic control on sex and age-specific vital rates of the populations. The importance of this study lies in the fact that, to our knowledge, it is the first comparative study of SEM and GSEM models in population dynamics. The analysis of our dataset illustrates some advantages in using GSEMs for discrete responses. SEMs are more flexible and have more parameters than GLMs and may better fit the data of interest. We believe that past work should be reviewed in the light of the results obtained here. Specifically, the results from studies using LMs should be considered with great caution, particularly in those cases where assumptions were clearly violated and transformations to normalise non-normal variables were applied. Finally, it is important to stress that the use of GSEMs can be extended to other behavioural and ecological contexts characterised by non-normal distributions of variables.

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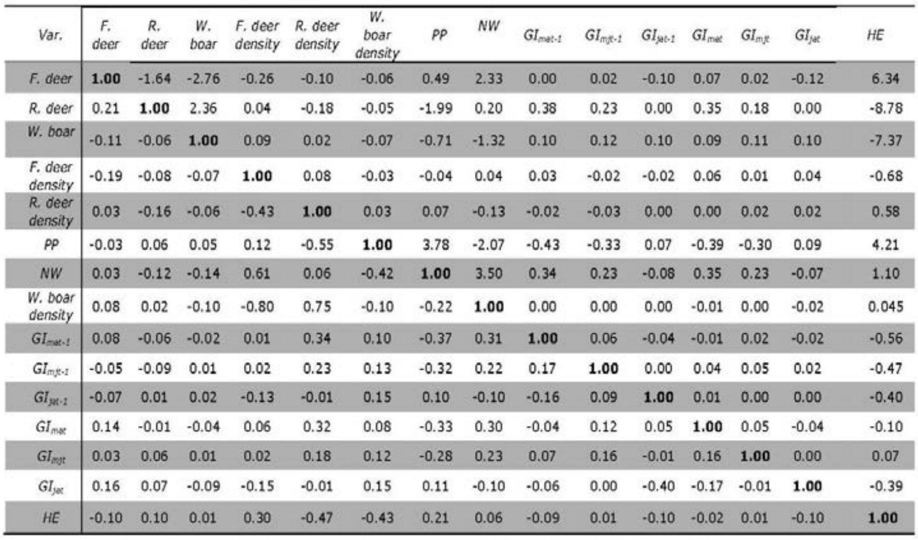
Supporting Information

S1 Table

Variance-covariance and correlation matrix used in SEM and GSEM for FCH and MDH models.

Table S1. Measured variance-covariance/correlation matrix in a data set consisting of 104 observations.



Var.	*F. deer*	*R. deer*	*W. boar*	*F. deer density*	*R. deer density*	*W. boar density*	*PP*	*NW*	GI_{mat-1}	GI_{mjt-1}	GI_{jat-1}	GI_{mat}	GI_{mjt}	GI_{jat}	*HE*
F. deer	**1.00**	-1.64	-2.76	-0.26	-0.10	-0.06	0.49	2.33	0.00	0.02	-0.10	0.07	0.02	-0.12	6.34
R. deer	0.21	**1.00**	2.36	0.04	-0.18	-0.05	-1.99	0.20	0.38	0.23	0.00	0.35	0.18	0.00	-8.78
W. boar	-0.11	-0.06	**1.00**	0.09	0.02	-0.07	-0.71	-1.32	0.10	0.12	0.10	0.09	0.11	0.10	-7.37
F. deer density	-0.19	-0.08	-0.07	**1.00**	0.08	-0.03	-0.04	0.04	0.03	-0.02	-0.02	0.06	0.01	0.04	-0.68
R. deer density	0.03	-0.16	-0.06	-0.43	**1.00**	0.03	0.07	-0.13	-0.02	-0.03	0.00	0.00	0.02	0.02	0.58
PP	-0.03	0.06	0.05	0.12	-0.55	**1.00**	3.78	-2.07	-0.43	-0.33	0.07	-0.39	-0.30	0.09	4.21
NW	0.03	-0.12	-0.14	0.61	0.06	-0.42	**1.00**	3.50	0.34	0.23	-0.08	0.35	0.23	-0.07	1.10
W. boar density	0.08	0.02	-0.10	-0.80	0.75	-0.10	-0.22	**1.00**	0.00	0.00	0.00	-0.01	0.00	-0.02	0.045
GI_{mat-1}	0.08	-0.06	-0.02	0.01	0.34	0.10	-0.37	0.31	**1.00**	0.06	-0.04	-0.01	0.02	-0.02	-0.56
GI_{mjt-1}	-0.05	-0.09	0.01	0.02	0.23	0.13	-0.32	0.22	0.17	**1.00**	0.00	0.04	0.05	0.02	-0.47
GI_{jat-1}	-0.07	0.01	0.02	-0.13	-0.01	0.15	0.10	-0.10	-0.16	0.09	**1.00**	0.01	0.00	0.00	-0.40
GI_{mat}	0.14	-0.01	-0.04	0.06	0.32	0.08	-0.33	0.30	-0.04	0.12	0.05	**1.00**	0.05	-0.04	-0.10
GI_{mjt}	0.03	0.06	0.01	0.02	0.18	0.12	-0.28	0.23	0.07	0.16	-0.01	0.16	**1.00**	0.00	0.07
GI_{jat}	0.16	0.07	-0.09	-0.15	-0.01	0.15	0.11	-0.10	-0.06	0.00	-0.40	-0.17	-0.01	**1.00**	-0.39
HE	-0.10	0.10	0.01	0.30	-0.47	-0.43	0.21	0.06	-0.09	0.01	-0.10	-0.02	0.01	-0.10	**1.00**

S1 Appendix: Mplus codes used to generate SEM and GSEM.

TITLE: SEM

```
x_daini     y1
x_capri     y2
x_cingh     y3
r_daini1    y4
r_capri1    y5
```

```
r_cingh1      y6
pineta2       y7
boschi_qm2    y8
gima12        y9
gimg12        y10
gila12        y11
gima2         y12
gimg2         y13
gila2         y14
n_cacciate    y15
habitat       f1
clima1        f2
climat_1      f3;
DATA:
        FILE = C:/Users/ Desktop/ProvaMplus/dati1_rid6.csv;
VARIABLE:
        NAMES = y1-y15;
        USEVARIABLES=  y1 y2 y3 y4 y5 y6 y7 y8 y9 y10 y11 y12
y13 y14 y15;

ANALYSIS:
        ALGORITHM=INTEGRATION;
        INTEGRATION=30;
        TYPE=GENERAL;
        ESTIMATOR=MLR;
        STARTS=5;
```

```
MODEL:
        !* The metric of the factor is defined by fixing the factor
variance at 1*!
        y3 ON y15;
        y4 ON y1 ;
        y5 ON y1 ;
        y6 ON y1 ;
        y5 ON y2 ;
        y6 ON y2 ;
        y4 ON y3 ;
        y6 ON y3 ;

        f1 by y7* y8;
        f1@1;
        y4 ON f3;
        y5 ON f1 ;

        f2 by y12* y13 y14;
        f2@1;
        y5 ON f2 ;

        f3 by  y9* y10 y11;
        f3@1;
        y15 ON f3 ;
        y5 ON f3 ;

        f2 ON f1;
        f3 ON f1 ;
```

```
        y4 with y6@0;
        y4 with y5@0;
        y1 with y2@0;
        y2 with y15@0;
        y1 with y15@0;
        y1 with f1@0;
        y2 with f1@0;
        y15 with f1@0;
  OUTPUT:   TECH1  TECH3  TECH8 TECH4 TECH10
        SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
  SAVEDATA:
  FILE IS DATIECO.TXT;
  PLOT:  TYPE=PLOT3;
```

TITLE: GSEM

```
DATA:
FILE = C:/Users/ Desktop/ProvaMplus/dati1_rid6.csv;

VARIABLE:


        NAMES = y1-y14 u15;
        USEVARIABLES=  y1 y2 y3 y4 y5 y6 y7 y8 y9 y10 y11 y12
y13 y14 u15;
        COUNT= u15 (nbi);
```

```
  ANALYSIS:
        ALGORITHM=INTEGRATION;
        INTEGRATION=30;
        TYPE=GENERAL;
        MCONVERGENCE=0.01;
        ESTIMATOR=MLR;
        STARTS=5;


  MODEL:
        !* The metric of the factor is defined by fixing the factor
variance at 1*!

        y3 ON u15;
        y4 ON y1 ;
        y5 ON y1 ;
        y6 ON y1 ;
        y5 ON y2 ;
        y6 ON y2 ;
        y4 ON y3 ;
        y6 ON y3 ;

        f1 by y7* y8;
        f1@1;
        y4 ON f3;
        y5 ON f1 ;

        f2 by y12* y13 y14;
```

```
    f2@1;
    y5 ON f2 ;

    f3 by  y9* y10 y11 ;
    f3@1;
    u15 ON f3 ;
    y5 ON f3 ;
    y4 ON f3 ;

    f2 ON f1;
    f3 ON f1 ;

    y4 with y6@0;
    y4 with y5@0;
    y1 with y2@0;
    y1 with f1@0;
    y2 with f1@0;

OUTPUT:   TECH1  TECH3  TECH8 TECH4 TECH10
    SAMPSTAT STANDARDIZED  RESIDUAL CINTERVAL;
SAVEDATA:
FILE IS DATIECO.TXT;
PLOT:  TYPE=PLOT3;
```

CONCLUSIONS

In the last years statistical methods in ethology and ecology have been improved a lot with the introduction of GLM and GAM allowing ecologists and animal behavior students greater flexibility to test working hypotheses.

Now another revolution is developing with the adoption of Structural Equation Models, which may open the way to substitute correlative with causal analyses, by developing explicit models of the hypothesis of interests.

A detailed discussion of SEM has been given, for instance, by Shipley (2000) and, specifically for ecology, by Pugesek et al (2003). However in many applications SEM cannot be used because it is based on normal theory while natural processes are often more complex and can be better described by other distributions. Further many variables of interest are ordinal or counts.

Thus a new statistical theory has been developed in the last years for the application of Generalized SEMs, which relaxes many of the statistical assumption of SEMs. For the first time I develop here a comparison between GLM, SEM and GSEM and show limits and advantages of the different techniques and give readers guidelines for the use of GSEM, since an established methodology is yet missing.

First I demonstrate the use of GSEM by analyzing a set of data on male behavior in a lek of fallow deer. These data are particularly challenging: distributions are not at all normal and field measurements are affected by errors and biases. Improvement in data analysis is a contribution to the study of sexual selection in lekking vertebrates.

The data set collected at Castelporziano on the mating behaviour of fallow bucks represents a typical example of the many studies performed on the leks of this species (Clutton-Brock et al. 1988; Fiske et al. 1998; Focardi & Tinelli 1996b; Apollonio et al. 2014) and other species of vertebrates (Sardell et al. 2014; Fiske et al. 1998; Kokko et al. 1998). These behavioural studies are important not only to identify the proximate causes of mate selection, but also for determining the intensity of sexual selection and understanding the evolution of exaggerated traits in males.

A literature review (cfr. S2 Text and S2 Table) allowed us to select the more popular methods used in previous research and to contrast them with innovative GSEMs. The use of the same dataset to compare different statistical methodologies is useful for evaluating their relative efficiency in data fitting. In general, LMs appear to be severely biased, and although GLMs may improve the reliability of the results, they overlook several important effects and the estimated coefficients still have low precision, which severely jeopardizes their predictive capacity. It is worth stressing that data transformation is not appropriate to normalize data distribution, since results appear extremely sensitive to the specific function used. This problem is exacerbated by the large number of zeros in the distribution of male copulatory success.

The introduction of GSEMs in the analysis of lek mating appears to represent a relevant leap ahead in the field. Our study provides evidence of several advantages of GSEMs compared to GLMs. First, the collinearity of predictors is no longer a nuisance provided that an appropriate measurement model is built, so we save part of the information collected in the field, which is usually lost in GLMs to reduce variance inflation (Zuur et al. 2010). Second, GSEMs are a flexible tool since they allow contrasting different causal models (e.g. using AIC, BIC, or other fit indexes) which must be formulated *a–priori*. In comparison to both LM and GLM, a proactive model formulation improves the awareness of the biological significance of the mechanism to be tested and allows scholars to modify a basic theoretical construct by introducing specific paths which are known or thought to be relevant in each particular study condition. This feature of SEMs allows us to include both general theoretical statements and specific conditions in the same model, which are then evaluated together. The publication of the variance-covariance matrix has the advantage of allowing other scholars to replicate the results easily and to propose different theoretical models pertinent to the system of interest, and in doing so, improve the transparency of the research and the full reproducibility of the results. However the availability of rough data can be useful to adjust the standard errors. Finally, SEM/GSEM help to control for measurement errors, a much neglected flaw in most quantitative analyses.

GSEM represents a bridge between the descriptive approach developed in LM and GLM and experimental tests with manipulative treatments; indeed the consistency of alternative causal paths can be tested, and when possible, the results can be used to develop more stringent experiments.

The importance of using GSEMs is well represented by the between-method comparisons reported in this study. First, we were able to show that, with respect to GLMs and even more to LMs, GSEMs suggest the potential influence of a larger number of predictors, in other words more informative models can be developed. This may have a strong impact on the interpretation of the study. For instance, both LMs and GLMs (except for the Poisson models) were unable to detect any effect of predictors referring to male dominance, which are however present, albeit with a small effect. Indeed in the literature, several authors were unable to detect these effects at all (e.g. Rintamaki 2001; Kervinen 2012; Apollonio et al. 1989; Loyau et al. 2007).

The second relevant aspect of GSEMs is the increased precision of the estimates of the regression coefficients. The analysis of residuals in GSEMs and GLMs confirmed that the former allowed a better fitting of the data than the latter.

While these results are not meant to disprove the available results about lek breeding of fallow deer based on linear models, the analysis of our dataset illustrates some advantages in using GSEMs for discrete responses. SEMs are more flexible and have more parameters than GLMs and may better fit the data of interest. Indeed, the formal definition of contrasting working hypotheses, such as FCH and MDH in this study, is illustrative of the potentiality of SEM for hypotheses testing. On the other hand, with respect to LMs and GLMs, SEM are data hungry and Shipley (2000) gives a rule of thumb to decide the number of parameters that can be safely estimated given a certain sample size.

The practical use of GSEM presents several difficulties. The main problem is that the likelihood of SEMs with latent variables is generally multimodal, and there is a need for a general algorithm to locate the global maximum. Moreover, the algorithm sometimes does not converge to a proper solution and this usually suggests that the model is not identifiable (at least in some parts). A partial remedy is to include reasonable identifiability constraints. In path analysis or with GLMs, the problems of non-convergence are generally absent.

One drawback that may limit a wider diffusion of GSEM is that the possibility of modelling non-normal variables is not yet implemented in widespread statistical packages, such as SAS, R, or S-plus. In this paper, GSEMs have been implemented in Mplus and STATA. We support the importance of using both packages, because they present complementary advantages and disadvantages.

The analyses in this thesis were developed under a frequentist approach. A Bayesian analysis of our data with GSEM is outside the scope of the present study and would require further research especially as far as the choice of priors is concerned. For an introduction to Bayesian SEMs see Kaplan & Depaoli (2012).

The importance of this study lies in the fact that, to our knowledge, it is the first comparative study of SEM and GSEM models. We believe that past work should be reviewed in the light of the results obtained here. Specifically, the results from studies using LMs should be considered with great caution, particularly in those cases where assumptions were clearly violated and transformations to normalise non-normal variables were applied. Interestingly, Grace et al. (2016) analysed the species richness-productivity relationships using SEM and showed that an integrative model has an higher explanatory power than traditional linear models, since SEM allows us to integrate competing hypothesis into a single model. Furthermore, SEMs help to solve the Simpson's paradox (Pearl et al. 2016). Finally, it is important to stress that the use of GSEMs can be extended to other behavioural and ecological contexts characterised by non-normal distributions of variables. SEMs are getting traction in behavioural studies and in ecology. Thus, GSEM can find wider and wider opportunities for application. In particular, the possibility of using SEMs to test hypotheses in competition and investigate both remote and proximate effects is of particular interest in ecological and evolutionary studies. The present study can therefore stimulate the application of GSEM to different study cases.

Traditionally the analysis of reproductive strategies in ungulates where females do not receive benefits from males, except genes, is focused on male mating tactics (see Lombardi et al. 2017 and references therein) since females are believed to be especially constrained by the resources used for rearing and protecting offspring (Clutton-Brock et al. 1988, Clutton-Brock et al. 1996, Ciuti et al. 2006). A small number of papers have dealt with female mating tactics in lekking ungulates (Clutton-Brock et al. 1989, McComb and Clutton-Brock 1994, Bro-Jørgensen 2002).

This thesis elucidates several open questions which were not dealt with in previous works relative to lek mating. The present analysis showed that female fallow deer adopt different tactics of mate choice in a lek and that variations in the mating tactics are associated with female experience and costs: females, who were either less experienced or incurred higher travel costs more often adopted indirect forms of mate selection when compared to adult females residing near the lek.

The large number of visits to the lek recorded in this study for some of the females was never observed before in other fallow deer leks (Apollonio et al. 2014, where however young females were not investigated). This difference may be attributed to: (1) a different study area size (Castelporziano with only one lek is 1.5 times larger than San Rossore where two leks have been observed, Apollonio et al. 2014), (2) hypothetical differences in population density and (3) in male dominance structure which affects the number of female visits (Apollonio et al. 1989).

The aim of this work is to fill this gap and to assess condition-dependent variations in female tactics in the lekking population of Castelporziano (Italy). In particular, we investigated three indirect selection mechanisms: i) *aggregation*, when females join an already formed group; ii) *copying*, when females copy the mate choice of other females and iii) *territory choice*, when females select a territory where many copulations had occurred previously.

An original finding of this paper is that younger females remained longer in the lek, performed more visits, were observed in a higher number of territories and longer in the vicinity of bucks than adults and returned to the lek also after copulating, Younger (1-2 year old) females were also the only ones to be recorded copulating more than once, however our limited sample (3 out of 29 tagged females) does not allow us to generalise this observation, and besides, a previous study found that fallow deer polyandry is not related to female age in a non-lekking population (Briefer et al. 2013). Overall, this apparently inefficient behaviour might be a by-product of their lack of experience and thus it can act as a learning occasion. Further, young females have no fawn at heel and their movements are less constrained than the ones of adult does (Ciuti et al. 2006). Multiple visits could favour mate quality assessment (Apollonio et al. 2014), however the young females did not arrive earlier than older ones, albeit males are already present and available to be assessed. We might therefore deduce that young females are mainly interested in observing the behaviour of other females at lek and in fact they were characterised by a later copulation date. The same was also observed by Farrell et al (2011) in a non-lekking population. Nevertheless, we found no evidence of a higher probability of copying in young females. A later copulation date of younger females could be explained by a difference in reproductive physiology between young and adult does (Mattiello 1994), or females of different age might respond differently to social stimuli: females can, in fact, adjust the timing of oestrus to maximise the possibility to mate with the preferred male (Komers et al. 1999). As a consequence, when compared to adult females, young females could be ready to make their mate choice only after a longer assessment of males at lek.

Despite the time spent at lek, it appears that young females are not able to select the highest rank bucks. Probably younger females are not able to identify high-ranking bucks because of their inexperience in assessing male physical features or in recognizing visual and olfactory marking activities that are supposed to be an important signalling of male reproductive status (Stenström et al. 2000). Also in a non-lekking population, younger females were found to mate on average with bucks of lower rank than adult ones (Farrell et al. 2011), indicating that this inability of young females is widespread in fallow deer populations regardless the adopted mating system.

Females living far away compensate for higher costs by spending less time, visiting less often and arriving later to the lek than near females. Despite these constraints, there is no difference between farther and near females with respect to the number of visited territories and time spent in the vicinity of bucks, as well as in copulation date. Farther females appear to use *aggregation* more than near females. Experimental studies showed that female fallow deer are attracted un-specifically by female groups (McComb and Clutton-Brock 1994) and these authors argued that this behaviour is probably useful to reduce harassment by immature males and that it allows females to copy the mate choice of others does (Clutton-Brock et al. 1988; Wiley, 1991). However we did not find evidence for a higher probability of *copying* or *territory choice* in farther females, therefore *aggregation* is likely to represent a cheap proxy for high ranking bucks, thus allowing to narrow the direct assessment only to the small number of males with a harem. The final outcome is that the reproductive success is independent on distance, suggesting that farther females are more efficient than close females. Surprisingly, younger females when compared to older ones, are not more likely to be observed together with other females. This observation confirms that young females are not as efficient in mate selection as adult does.

The fact that far and near females exhibits the same reproductive success mating with same rank bucks suggest a mechanism for the origin of multiple arenas which are present in some populations (Apollonio et al. 2014). As far as the increased costs of a longer distance between the home range and the lek can be buffered by modifications in female behaviour, as we have shown in this paper, the whole population can congregate in a single lek, but we expect that when farther females are no more able to compensate travel costs they are more likely to congregate in a different but nearer arena. We believe that in the case of Castelporziano, we are at the limit of a size where a single lek can be present; indeed the farthest females of our samples were in a distance class of 8.5 km and not every year were able to attain the lek. The critical distance threshold is not expected to be the same in every study area since travel costs depend on a complex way of topography, vegetation type, physical barriers (roads, channels) presence of predators, hunting and disturbance.

Mate choice by females based on the selection of successful territories is widespread in lekking species (fallow deer: Apollonio et al. 1990; Uganda kob: Balmford 1991; blackbuck *Antilope cervicapra*: Isvaran and Jhala 2000; black grouse *Tetrao tetrix*: Kokko et al. 1999; great snipe *Gallinago media*: Sæther et al. 2005). This is because the location of a territory within the lek can be an honest signal of male quality, enabling less costly mate sampling and potentially more accurate mate choice than direct female mate assessment (Kokko et al. 1999), else females may select territories partly using environmental cues, regardless the rank of the male (Sæther et al. 2005). A novel result in our study is that younger females are more likely to select a successful territory (*territory choice*) than older females. They could use this tactic to increase the probability of mating with a high quality buck but this study has shown that such an approach is on average unsuccessful, probably because of the rapid turnover of bucks on better territories. Thus, the increase in male reproductive variance later in the season can be explained by young females adopting a tactic of *territory choice* and so mating mostly after the *Peak*.

We were unable to detect a different amount of *copying* in young or in farther females. This observation had been unexpected because previous studies have suggested that copying can be an effective tactic for cost-reducing and increasing precision (Gibson and Höglund 1992). This finding concords with McComb and Clutton-Brock, (1994) who found aggregation, but not copying, in female fallow deer under experimental conditions. The review by Vakirtzis (2011) evidences that the presence of copying is variable in lekking species. Pruett-Jones (1992) used a theoretical game to show that it is convenient for females to copy each other’s mating decisions only when the costs of searching are not negligible. This is not the case of Castelporziano, an undisturbed area with no obstacles to animals’ movement. Moreover, Gibson and Höglund (1992) argued that the benefits of imitation increase if the individuals that mate first are the ones with the greatest experience so we have hypothesized a more frequent *copying* in younger does. Losey et al. (1986) have shown that the benefit of copying increases with the number of “peeks” at lek and indeed young females stay longer at the lek than adults. However, younger females can be deterred to copy mate choice by a rapid male turn-over in territories. On the other hand, farther females cannot spend enough time in the lek to get enough peeks. A further argument which can explain the lack of differences in copying among near and farther females is males’ sperm depletion, more likely in bucks who have mated earlier (Gibson and Höglund 1992). Insemination failure is a problem especially for farther females, which would incur in higher costs than near ones if obliged to come back to the lek.

Our results suggest, but do not prove, that adult near females probably perform a direct choice more than farther females. Indeed they stay longer, visit more often and arrive earlier. We can explain this pattern assuming that direct choice is especially complex and requires a careful assessment of both physical features and behaviour of bucks. In other species, direct choice is used more than indirect tactics. This is the case of the great snipe (Sæther et al 2005) and of the non-lekking pied flycatcher *Ficedula hypoleuca* (Slagsvold and Viljugrein 1999). Since fallow deer exhibit a strong plasticity in the mating system (Langbein 1999) which also depends on population density (Apollonio 1989) we can expect that direct choice may vary among populations and be more frequent in low density populations. This study demonstrated the condition-dependent variation in female mating decisions in an ungulate lek, a crucial issue to improve the understanding of mammalian mating systems (Bro-Jørgensen 2011) and already evidenced in a wide range of taxa (Cotton et al. 2006). Our results can thus contribute to further clarifying the basis of co-evolution of mating strategies in both sexes and, eventually, the evolution of ungulate leks.

In synthesis the results show that female fallow deer, which are less experienced (young) and/or incur higher travel costs (home range far from the lek), adopt indirect forms of mate selection more often than adult females or females residing near the lek. In particular, younger females remained longer in the lek and in the vicinity of bucks than adult ones, and returned to the lek after copulation. However, despite the time spent at the lek, young females were not able to select highest-rank bucks, and relied on *territory choice* more often than adult does. Farther females visited the lek less frequently and arrived later than near females, but they were seen more often within female groups. Surprisingly, we did not find a different amount of *copying* in young or in farther females. Our results can contribute to clarifying the co-evolution of mating strategies of both sexes in ungulate leks.

Empirical studies of ecological communities need to account for a large number of reciprocal effects among the components of the studied ecosystem. It is of the uppermost interest to disentangle all these relationships, to discriminate causal effects from spurious correlations and deal with observational errors, which, in studies performed under natural conditions may be large and have the potential to bias our conclusions. To overcome these problems, the use of structural equation models (SEM) is becoming more and more widespread in ecology. Here we review the pertinent literature and discuss pros and cons of this statistical methodology. Further, we show the application of SEM to the analysis of a 100-years-long time series relative to the abundance of three sympatric species of wild ungulates (roe deer, wild boar and fallow deer).

In this thesis I presented a long-term analysis of the population dynamics of a community of ungulates living in a Mediterranean climate and integrates the research presented by Imperio et al. (2012). Data validation (Imperio et al. 2010) indicated that the effects described in this paper were not due to the hunting regime carried out in the Preserve. We investigated the role of intra- and inter-specific competition on the dynamics of three ungulate species, together with the effects of both environmental and climatic factors. The main hypotheses tested in this work were that 1) the complexity of the ungulate community influences intra- and inter-specific interactions; and 2) in Mediterranean ungulate communities, exposed to relatively mild climate fluctuations, trophic interactions such as density dependence, competition and facilitation have stronger effects than environmental controls such as climate (explored by a SEM and GSEM approach). The results of all analyses confirmed that intra-specific competition was a main ingredient in the regulation of the growth rates within the ungulate community at Castelporziano, in keeping with the view that density-independent regulation is more important in those populations facing strong climatic fluctuations (Post 2005). Inter-specific competition, appeared to be generally of lower importance, although evidences of interactions among species were found. Perhaps the most relevant impact was that of fallow deer on the dynamics of roe deer, presumably as a consequence of the very high fallow deer density at that time. Results of the GSEM model suggest that the observed inverse correlation between fallow and roe deer densities was not a side effect of spurious correlations with unknown variables but, instead, was due to a direct competition between the two species (Putman 1996). This finding is consistent with the available information on inter-specific competition in Mediterranean environments (Focardi et al. 2006; Ferretti et al. 2008). Other effects included the negative influence of wild boar on fallow deer, the negative effect of red deer on wild boar, and the apparent positive effect of roe deer on red deer and wild boar. The interpretation of these latter effects is however less straightforward. Results of this study suggest that the negative effect of wild boar on fallow deer was probably indirect, related to the impact of selection hunting to control wild boar. The higher the wild boar density, the more intense presumably was the hunting pressure, with a larger number of hunting drives affecting also other ungulates such as fallow deer. On the contrary, the apparent facilitation exerted by roe deer could be caused by a spurious correlation with a third unknown variable. A pattern of positive correlation between wild boar and roe deer was also observed by Mysterud et al. (2007) in Poland, but it appears theoretically difficult to imagine which mechanisms were operating. A possible explanation is that both species were positively influenced by acorn production: since the two species are polytocous, they could be in the best biological conditions to exploit the window of opportunity represented by masting.

The hunting effort seems to play an important role in determining the growth rate of wild boar as opposed to what was found by Imperio et al. (2012) in which they did not find any association between HE and any of these species, except for fallow deer in the post-removal period.

Habitat composition and climate have been shown to affect to some degree the three species. However, these effects had a secondary importance with respect to intra- and interspecific competition in regulating the ungulate populations studied here. Habitat factors entered the models in terms of latent variable 'habitat quality' in the SEM and GSEM model. Unfortunately, no information on actual resource availability (e.g. mast production) was available. As for climate, very wet conditions in May–June appeared to play a negative role for all deer species, probably being associated with insurgence of thermoregulatory problems in fawns (Van Moorter et al. 2009). On the contrary, water availability during the spring of the previous year had a positive effect on both fallow and roe deer. This effect is presumably due to increased primary productivity during moist springs, which in turn improves body conditions in young females and thus enhances their fecundity (Gaillard et al. 1992). In fact reproductive success of roe deer depends mainly on environmental conditions, in spring-summer (McLoughlin et al. 2007; Pettorelli et al. 2005). Neither of these factors, however, had any effect on wild boar dynamics. This result is consistent with observations on modern data, showing a weak effect of climate on this species and mainly on subadult males (Focardi et al. 2008). Finally, summer drought has been shown to play an almost insignificant role in the dynamics of the studied species. In general the summer Gaussen index values fluctuated widely during the entire span of the study period but no clear downward trend was observed. However, our data set ends in 1986, whereas the mean annual temperature started to grow more rapidly afterwards (Brunetti et al. 2006). The importance of climatic factors, and in particular of summer droughts, could thus grow in coming years, as climate change projections indicate an expected increase of aridity and of heat waves in most Mediterranean regions (Gao & Giorgi 2008). The results of this study provided also important information on the population dynamics of individual species. Fallow deer appeared not to be much influenced by the other herbivores. This independence is presumably linked to its trophic position (between intermediate and roughage eaters) in the community of ungulates (Hofmann 1989), as fallow deer is able to exploit low quality browse but also to benefit from the flushing of grasses during some periods of the year. For this species, the final regulatory mechanism was represented by a combination of climatic factors and density dependence, together with a positive effect of the amount of pine plantations.

Direct density-dependence explained the growth rate of roe deer at a much lower extent, being influenced by habitat factors (in particular by a negative impact of pine woods), climate and competition with fallow deer. These observations are consistent with life history theory of ungulates. Density dependence in roe deer is well established even in south-European populations (Focardi et al. 2002; Kjellander et al. 2006), but in previous study cases roe deer had no competitors. At Castelporziano, density dependence was operating but it was accompanied by other relevant regulatory processes.

The wild boar population appeared to be scarcely linked to the other ungulates. Theoretical considerations (Focardi et al. 2008) suggest in fact that the demography of this suid is quite different from that of the other species.

This study was made possible by the availability of an exceptionally long data set: because of the peculiar management model of Castelporziano, bag records and other types of information were carefully conserved for more than one century. This allowed for estimating harvest density (Imperio et al. 2010); in addition, climate records and the availability of unusually old aerial photographs allowed to carefully track habitat variations in the area. We have to note that an important drawback of the data analyzed here was the lack of information on population structure.

In addition, the data were characterized by a high level of noise and by several years during which population sampling was poor, a problem that was addressed by the use of a SSM to filter the data (Imperio et al. 2012) prior to the SEM and GSEM analyses. The results presented here should be used to formulate working hypotheses to be verified with recent data, collected in a more controlled way, in order to disentangle the role of competition and climatic control on sex and age-specific vital rates of the populations. The importance of this study lies in the fact that, to our knowledge, it is the first comparative study of SEM and GSEM models in population dynamics. The analysis of our dataset illustrates some advantages in using GSEMs for discrete responses. SEMs are more flexible and have more parameters than GLMs and may better fit the data of interest. We believe that past work should be reviewed in the light of the results obtained here. Specifically, the results from studies using LMs should be considered with great caution, particularly in those cases where assumptions were clearly violated and transformations to normalise non-normal variables were applied. Finally, it is important to stress that the use of GSEMs can be extended to other behavioural and ecological contexts characterised by non-normal distributions of variables.

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