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UNRAVELING THE NATURE OF INDIVIDUAL RECOGNITION BY ODOR IN HERMIT CRABS

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Abstract—Individual recognition is a key element in the social life of many invertebrates. However, most studies conducted so far document that several species are capable of a "binary" discrimination among conspecifics, but not of a "true individual recognition." Our objective was to learn more about the mechanisms that underlie individual recognition by odor in hermit crabs by individuating some of its properties. Using *Pagurus longicarpus* Say 1817 as a model species, we conducted four series of experiments in which the response of every test crab (the "receiver") to the different odor treatments (emitted by a "sender") was evaluated from its investigative behavior toward an empty, high-quality shell. After having excluded the possibility that crabs chemically recognize familiar/unfamiliar shells and/or shells of high/low quality, we explored whether the receivers discriminate odors from two familiar senders and whether this discrimination also occurs with unfamiliar crabs. We also asked whether crabs form an association between the odor of a familiar sender and some of its relevant attributes, i.e., rank, size, and shell quality. Finally, the shells inhabited by familiar individuals were manipulated to modify the association between odor and shell quality. Results showed that: (1) there is no odor specific of a rank; (2) individual crabs discriminate their own odor from the odor of other individuals; (3) they can chemically discriminate between larger crabs inhabiting higher-quality shells and smaller crabs inhabiting lower-quality shells, provided that these crabs are familiar to them; (4) they associate the odor of an individual crab with the quality of the shell it inhabits; and (5) this association quickly changes when social partners switch to shells of different quality. These results indicate that the nature of chemical recognition in *P. longicarpus* is more refined than a simple binary

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system. The receiver appears able to associate a type of information from the sender with memories of past experiences, therefore suggesting the hermit crab's potential for relatively high-order knowledge about conspecifics.

Key Words—Individual recognition, odors, dominance hierarchies, hermit crabs, *Pagurus longicarpus*.

INTRODUCTION

Individual recognition is a key element in the social life of many organisms, where it can play an essential role in the structure and stability of a number of behavioral networks, such as dominance hierarchies, territorial defense, competitive aggression, pair bonds, mate selection, and kin favoritism (reviewed in Zayan, 1994). The most detailed information available in the literature concerns the recognition of vocal signals by birds (e.g., Falls, 1982). Many studies have demonstrated the ability of nonhuman mammals to discriminate individuals by the use of chemicals (e.g., Halpin, 1980, 1986; Brown et al., 1990; Hurst et al., 2001). Particularly in the last few years, considerable effort has been directed at defining in vertebrates the processes of identification and recognition (Beecher, 1989): at the cognitive analysis level, questions have been addressed on the nature of individual representation exhibited by a handful of vertebrate taxa and on the evolutionary pathways leading to high-order knowledge about individuals (e.g., Johnston and Bullock, 2001).

To date, a relatively small body of literature exists that analyzes these issues in invertebrates (see, e.g., Leonard et al., 1974 in *Drosophila* spp.; Barrows et al., 1975 in halictid sweat bees *Lasioglossum zephyrum*; Liechti and Bell, 1975 in the cockroach *Byrsotria fumigata*; Linsenmair and Linsenmair, 1971 in the desert wood louse *Hemilepistus reaumuri*). Even fewer studies exist that have advocated pheromones as the basis of individual recognition in aquatic invertebrate species (Wickler and Seibt, 1970 in the clown shrimp *Hymenocera picta*; Johnson, 1977 in the banded shrimp *Stenopus hispidus*; Caldwell, 1985 in the mantis shrimp *Gonodactylus festae*; Karavanich and Atema, 1998 in the American lobster *Homarus americanus*).

Among other aquatic invertebrates, hermit crabs are optimal model organisms to investigate the mechanisms of chemically mediated individual recognition. The ability to recognize individuals in *Pagurus bernhardus* (Hazlett, 1969) or to discriminate familiar from unfamiliar conspecifics in *P. longicarpus* (Gherardi and Tiedemann, 2004a) was assumed to be a means to maintain stable hierarchical relationships. In fact, dominance hierarchies (Allee and Douglis, 1945; Winston and Jacobson, 1978) seem not to be laboratory artifacts, but they may develop in the small temporary aggregations of hermit

crabs that often form in tide pools (Scully, 1978), mostly around gastropod predation sites (Rittschof, 1980a).

A number of behavioral studies have shown that olfaction in hermit crabs can mediate a form of chemical recognition. Several species display adaptive behaviors when exposed to odors that signal shell availability (e.g., Rittschof, 1980a; Rittschof et al., 1992; Rittschof and Hazlett, 1997; Gherardi and Atema, 2005a), and chemical cues in the medium affect investigatory responses toward shells occupied by conspecifics (Hazlett, 1996a,b; Rittschof and Hazlett, 1997; Hazlett, 2000). Recently, Gherardi and Tiedemann (2004b) showed that *P. longicarpus* spends more time investigating an empty shell in the presence of odors released by unfamiliar, rather than familiar, conspecifics.

Based on these premises, our objective here was to learn more about the mechanisms that underlie chemical individual recognition in hermit crabs by using *P. longicarpus* as a study species. After having excluded the ability of crabs to recognize shells by odor, we explored whether they discriminate between two familiar individuals of different rank, size, and shell quality and whether this ability is expressed also toward unfamiliar crabs. Experiments have documented a binary discrimination between familiar and unfamiliar individuals, but have failed in demonstrating a recognition of one out of many, known individuals (Gherardi and Tiedemann, 2004a), even if provided suggestions of its potential (Gherardi and Atema, 2005b). Our experimental design here showed that the number of the individuals *P. longicarpus* can recognize is wider than previously thought. Then, two general questions were raised. The first was whether hermit crabs form an association between the odor of a familiar conspecific and one of its relevant attributes (rank, size, or shell quality). In other words, what does the odor of a crab mean to another individual? Finally, because the odor appeared to be associated with the high/low quality of the shell occupied by the "sender" crab (here defined as the crab releasing the odor, without any implication of signal selection for communication), we investigated the plasticity of this association by experimentally altering shell quality.

METHODS AND MATERIALS

Subjects, Collection, and Housing Conditions. The long-clawed hermit crab, *P. longicarpus* Say 1817, is common in shallow waters along the western Atlantic coasts of North America, from Nova Scotia south to eastern Florida, and in the northern Gulf of Mexico from the west coast of Florida to Texas (Williams, 1984).

Between July and August 2003, we hand-collected around 400 hermit crabs with the major chela width (CW) of 0.1–0.4 mm (corresponding to individuals

with shield length of about 4–6 mm) from Little Sippewissett salt marsh (Massachusetts, USA) during diurnal low tides. Immediately after capture, crabs were separated into small groups and transferred to the Marine Biological Laboratory in Woods Hole, where they were maintained in groups of up to 25 individuals in a temperature-controlled room (22°C) and under a natural 14-h light–10-h dark cycle. They were kept in separate 20-l holding aquaria containing constantly aerated seawater and fed with a diet of commercial shrimp pellets every third day. Water was changed weekly. After being used in experiments, crabs were released at the collection site.

Experimental Design. We conducted four series (A–D) of one to three related experiments (conditions). Each condition consisted of a 24-hr familiarization phase immediately followed by a test phase in which every test crab (the "receiver") was subjected to two to three different odor treatments in a random sequence. A preliminary experiment (experiment A) aimed at exploring whether hermit crabs chemically recognize familiar/unfamiliar shells (condition A1) and/or shells of high/low quality (condition A2). Then, we aimed at understanding in Experiment B whether crabs discriminate between two familiar conspecifics of different rank, size, and shell quality (condition B1) and whether this discrimination occurs also toward unfamiliar conspecifics with similar attributes (condition B2). In experiment C (with conditions C1, C2, and C3), we asked whether receiver crabs form an association between this odor and relevant attributes of the sender, i.e., its rank, size, and shell quality. Finally, experiment D was a continuation of C3, in which the shell inhabited by the sender was manipulated to modify the association between odor and shell quality. The logic behind our study is shown in the flowchart of Figure 1, and details of each experiment are given in Table 1.

Experimental Methods. All experiments and conditions were staged in opaque plastic bowls (10-cm diam), containing 160-ml unfiltered seawater at 22°C, illuminated during observations by a 75-W incandescent light, 50 cm above the water level. Observations were always conducted between 0900 and 1600 hr.

At least 10 d from the collection and 2 d before the beginning of experiments A–C, each crab was randomly assigned to one of the two or three conditions. We always used intact individuals (no missing limbs), and, for experiments B and C, we formed groups composed of three crabs ("trios"), using animals taken from separate holding aquaria to ensure that they had no memory of one another (in fact, crabs forget a familiar conspecific after 5 d of separation; Gherardi and Atema, 2005b). According to the major chela width, crab size was categorized as large, L (CW > 0.33 mm), medium, M (CW 0.23–0.33 mm), or small, S (CW < 0.23 mm). Sex was not noted because sex has been shown to exert no effect on agonistic interactions in this and other hermit crab species (Hazlett, 1966; Winston and Jacobson, 1978), at least during the

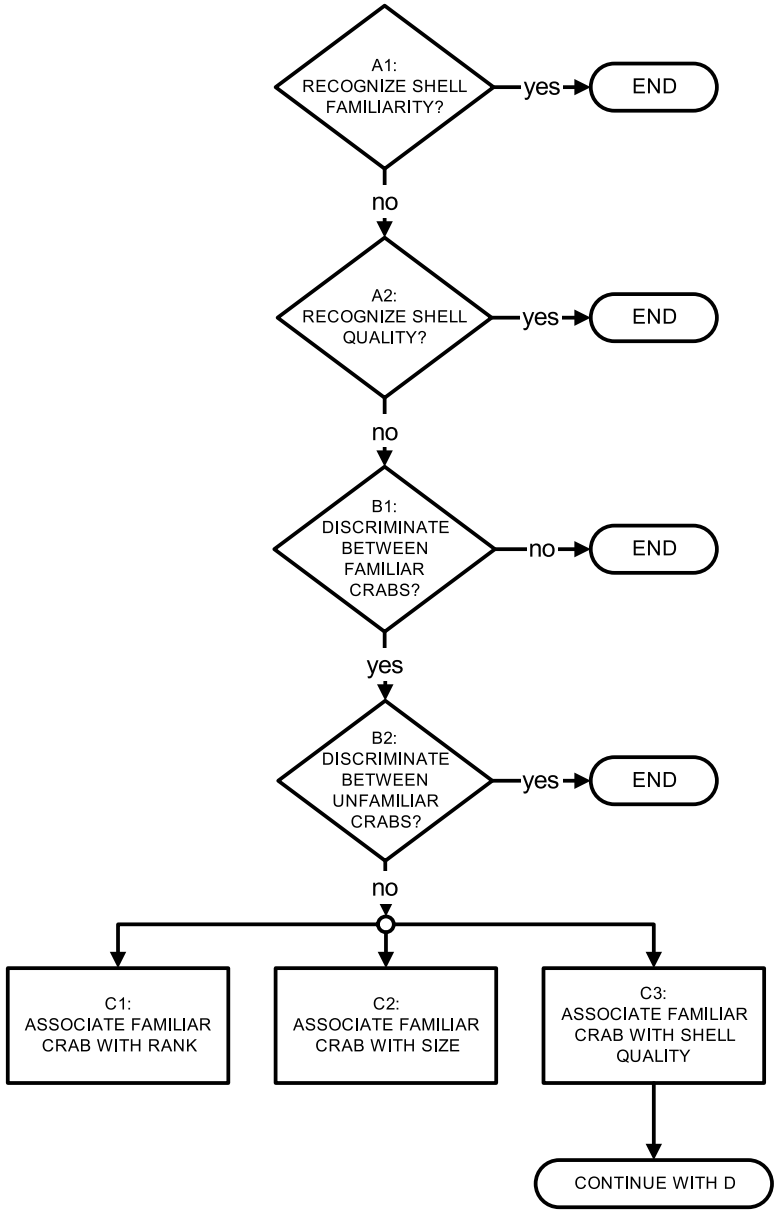

FIG. 1. A flowchart showing the sequence of questions addressed in this study. A1, A2, etc. denote experiments/conditions. Experiment D consisted of altering the association between familiar crab and shell quality.

TABLE 1. DETAILS OF THE EXPERIMENTS

Experiment/ condition	Test for	Receiver's attributes	Sender's attributes (A: shells; B–D: crabs)					
			Familiarity	Crab rank	Crab size	Shell size	Shell quality for the receiver	*n*
A1	Shell odor	Any crab	Yes/no				LTO, LTO	18
A2	Shell odor	Any crab	Yes				STO, LTO	17
B1	Familiarity	β (rank/size/shell quality)	Yes	α, γ	L, S	L, S	LTO, STO	26
B2	Familiarity	β (rank/size/shell quality)	No	α, γ	L, S	L, S	LTO, STO	21
C1	Rank	β (rank)	Yes	α, γ	M, M	M, M	OPT, OPT	15
C2	Size/rank	β (size/rank)	Yes	α, γ	L, S	M, M	OPT, OPT	13
C3	Shell quality/rank	β (shell quality/rank)	Yes	α, γ	M, M	L, S	LTO, STO	31
D	Plasticity	β (shell quality/rank)	Yes	α, γ*	M, M		STO, LTO	11

Experiment A evaluated the effect exerted on receiver crabs by the odor of shells with different familiarity and quality, and experiments B–D by the odor of crabs with different attributes (familiarity, dominance rank, size, and shell quality). The effect of the sender's odor was assessed from the investigatory behavior on a target shell performed by receiver crabs (any crab in experiment A and β crabs in experiments B–D). All the receivers were medium in their size and occupied medium-sized shells that were optimal for them. Asterisk denotes that individuals in D were the same individuals used in C3 but in shells of opposed quality, i.e., α and γ crabs of C3 occupying a STO and a LTO shell, respectively.
OPT: optimal shell size; STO: smaller-than-optimal shell size; LTO: larger-than-optimal shell size; L: large crab size and shell size; M: medium crab size and shell size; S: small crab size and shell size; α, β, and γ: dominance rank; *n*: sample size.

nonreproductive period (this species reproduces between October and May with a peak in autumn; Wilber, 1989). When crabs were tested in groups (experiments B, C, and D), the shells of every trio were marked by 0, 1, or 2 dots of permanent black ink to permit their identification. Individual hermit crabs were recognized by the length of their antennae and by slight differences in cheliped and pereopod color.

To make test animals as homogeneous as possible (for experiment A) and to obtain individuals that were similar (in C1 and C3) or different (in experiment B and in C2) for the quality of the occupied shell, crabs were extracted from their original shell by gently breaking it with a vise. Then, they were allowed to enter a new shell from a collection of five empty, undamaged, similarly sized shells that were prepared by collecting live periwinkle *Littorina littorea* (the dominant shell type used by the study population), boiling and removing the flesh, rinsing the shells several times in seawater, and air-drying them to remove odors of previous occupants (snail, epiphytes, etc.). Crabs were allowed 48-hr access to new shells, at which time they had ceased exploring and moving into new shells.

The size of the offered shells were changed in each experiment depending on the experimental protocol (see Table 1 and below). Shells were classified according to their length (measured from the base–apex axis in millimeters, SL) and to their adequacy for the body size of the inhabiting crabs. In fact, although hermit crabs can have preferences for various characteristics of shells, particularly in the case of the study species (Wilber, 1990), size is the most important determinant of shell selection.

Shells were categorized as L (SL > 18 mm), M (SL: 15–18 mm), and S (SL < 15 mm). The size of the optimal shell for a crab of a given size (OPT) was computed by regressing the equation $y = 37.9x + 7.3$, where y is SL and x is crab size (CW, in millimeters). This equation was obtained from a preliminary free-choice experiment in which every crab (of a total of 192) was allowed to choose among five empty shells of different size. Then, we defined as "larger-than-optimal" (LTO; and "smaller-than-optimal," STO) for a crab of a given size a shell whose size was about 10% longer (and shorter) than the size of the shell optimal for it.

Behavioral Assay: Crab Responses to a High-Quality Shell. The behavioral assay we used in the test phase was the investigative behavior shown by hermit crabs toward a novel shell that was about 10% longer than the optimal shell for the test crab's size (Gherardi and Tiedemann, 2004b). Notwithstanding its relatively large volume and heavy weight, this shell is highly attractive to *P. longicarpus*, as shown in a previous study (Gherardi, 2005). The cost of wearing a too large shell (e.g., the energetic costs of locomotion) seems to be overweighed in this species by a number of possible benefits. For instance, by accepting or even selecting an oversized shell, crabs may delay the need to find

larger shells to assure their growth and reproduction and gain some fighting advantages (Gherardi, 2005).

Tests were run in bowls containing 160-ml seawater that had been conditioned for an hour with the odor released by either shells (experiment A) or conspecifics (experiments B, C, and D). The experimental bowl was provided with an empty periwinkle shell placed with its apex upward, functioning as the target shell. This shell was prepared as described above for shell choice experiments; however, here, its aperture was blocked with a resin to avoid its occupation by the crab. Preliminary observations had shown that the resin and its odor had no effect on shell attractiveness.

Tests started by placing an individual (the receiver crab) into the bowl about 8 cm from the shell. Each receiver was subjected to two or three subsequent odor treatments, the sequence of which was varied systematically per crab. All hermit crabs of experiment A were used as receivers. In the other experiments in which we worked with trios, tests were conducted only on those crabs that had an intermediate score (hereafter defined β crabs) for dominance rank in experiments B and C1, for size (and rank, see below) in C2, and for shell quality (and rank, see below) in C3 and D.

For every odor treatment, the events occurring during 5-min observation and time were recorded on a voice tape and later analyzed to obtain: (1) latency in seconds (time until first shell investigation; when the test crab never investigated the shell, we arbitrarily assigned a time equal to 305 sec); (2) number of bouts of shell investigation; (3) total duration of shell investigation in seconds; and (4) total time spent in locomotion in seconds.

Experiment A: Recognizing the Odor of Empty Shells. Details for experiment A are given in Table 1. During shell choice, crabs were offered with shells optimal for their size. Then, they were kept isolated for 24 hr in bowls to become familiar with either one (LTO, A1) or two (one LTO and one STO, A2) empty, resin-blocked periwinkle shells. Treatments in the test phase were the odor from the familiar shell, as opposed to the odor from a novel shell (A1), and the odor from the LTO shell, as opposed to the odor from the STO shell (A2).

Experiment B: Discriminating the Odor between Familiar/Unfamiliar Conspecifics with Different Attributes. In this experiment, we investigated whether hermit crabs were able to discriminate by odor between two familiar (B1) or two unfamiliar (B2) conspecifics with different rank, size, and shell quality. Our purpose was to understand if crabs could chemically recognize more than one familiar individual while excluding the effects of rank odor. Trios were composed of α (large body size and large shells, OPT for the senders but LTO for the receivers), β (medium body size and medium shells, OPT for them), and γ (small body size and small shells, OPT for the senders but STO for the receivers) crabs. Other details are given in Table 1.

Familiarization started by placing three crabs in the experimental bowl and lasted 24 hr. The day after, we checked for shell switches that might have occurred overnight (none), and then we recorded the events taking place during 10-min observation; from these records, we evaluated the agonistic level of every trio from the number and the duration of fights. We defined as fights those interactions that started when one crab approached one or two rivals and ended when one or two opponents retreated to a distance greater than 3 cm and for at least 10 sec.

The dominance rank of an individual was defined from the relative number of wins. The winner was the opponent that did not retreat at the end of the interaction or that retreated after the other(s) withdrew into the shell. In the few instances in which the three crabs won the same number of interactions, we defined the ranks from the intensity of crab locomotion (i.e., the time spent exploring the experimental bowl). We never observed dominance reversal during any 10-min observation. Based on Winston and Jacobson's (1978) data, 24 hr were sufficient for the formation of a dominance hierarchy. In all groups analyzed, crabs that were classified as α, β, and γ for size and shell quality were also α, β, and γ, respectively, for rank. In the test phase, β crab behavior was analyzed in the presence of the odor released (1) by themselves (in both B1 and B2) or (2) by familiar (in B1) or unfamiliar (in B2) α and γ crabs.

Experiment C: Associating the Odor of Familiar Conspecifics with One Attribute. Because crabs seemed to discriminate between odors emitted by α and γ familiar conspecifics with different attributes, our aim was to investigate whether hermit crabs can associate the odor of the sender with one of its attributes. To this end, we analyzed crab behavior in the presence of odors from familiar conspecifics that differed for a single attribute (size in C2 and shell quality in C3), the other being equal. The results obtained from C2 and C3 were compared with C1, in which crabs differed for rank but had equal size and shell quality.

The familiarization phase was conducted as in experiment B. With only one exception in C3, crabs intermediate by size and by shell quality (i.e., β crabs in C2 and C3, respectively) were also β by rank. In the test phase, β crabs (the receivers) were presented with the odor of familiar α and γ crabs, which had the same size and shell quality as the receiver in C1, a different size (being either larger or smaller) but the same shell quality in C2, and the same size but either a LTO or a STO shell in C3 (see Table 1 for details).

Experiment D: Plasticity of the Association between Individual Odor and Shell Quality. We tested if crabs that had formed an association between the odor of a familiar conspecific and its shell quality would form a new association when the conspecific switched to a new shell of a different quality. In this experiment, we therefore investigated crab behavior when the familiar con-

specifics had been forced to occupy shells of poorer quality with respect to the shells they occupied previously.

We analyzed the results of 11 out of the 31 trios of C3, since five crabs died overnight and 15 shell switches occurred. Immediately after the test phase of C3, the shells occupied by α and γ crabs were gently broken with a vise, and crabs were forced to enter a novel shell that was STO for the former α crab and LTO for the former γ crab. After an hour of separation (not sufficient to forget former opponents; Gherardi and Atema, 2005b), we reconstituted the trios with the same individuals as in C3 but now with α in a low-quality shell and γ in a high-quality shell (see Table 1 for details). The new trios were then subjected to the familiarization phase and to the test phase as in experiment C.

Data Analyses. We applied nonparametric tests (Sokal and Rohlf, 1969; Siegel and Castellan, 1988) because the assumptions of normality of data and homogeneity of variance were not always met, and some measures taken represented ordinal data. We used Mann–Whitney *U* tests (statistics: *U* and *z* for samples >20) and Kruskal–Wallis one-way analyses of variance (statistic: *H*) to examine differences in the agonistic level reached by trios during the familiarization phase between/among the conditions of experiments B and C, respectively. We compared conditions for latency and for the other measures taken during the test phase using Wilcoxon matched-pairs signed-ranks tests (statistics: *T* and *z* for samples >25; experiments A, B, and D) and Friedman two-way analyses of variance (statistic: Fr; experiment C). When the null hypothesis was rejected after Friedman two-way analysis of variance, a multiple comparisons test (Siegel and Castellan, 1988) was used to determine which pairs of samples differed significantly. The test takes into account the correction of the α level for multiple comparisons (Siegel and Castellan, 1988). Text and figures provide medians and interquartile ranges (first–third quartiles). *P* values of less than 0.05 were considered statistically significant.

RESULTS

Experiment A: Recognizing the Odor of Empty Shells. Crab behavior in the test phase did not differ when water was conditioned with odor from either familiar or unfamiliar shells (A1) or from familiar shells of LTO/STO quality (A2; Table 2). Thus, crabs did not recognize familiar or high-quality shell attributes alone.

Experiment B: Discriminating Odor between Familiar/Unfamiliar Conspecifics with Different Attributes. In the familiarization phase, trios used for B1 and B2 displayed the same agonistic level (number of fights: $z = 0.86$, $P = 0.39$; duration of fights: $z = 1.44$, $P = 0.66$). This established that the results obtained

TABLE 2. STATISTICAL OUTPUTS OF EACH EXPERIMENT AND CONDITION FOR LATENCY, BOUTS OF SHELL INVESTIGATION, DURATION OF INVESTIGATION, AND TIME OF LOCOMOTION

Experiment/ condition	Latency		Shell investigation		Duration of investigation		Time of locomotion	
	T/*z*/Fr	*P*	*T*/*z*/Fr	*P*	*T*/*z*/Fr	*P*	*T*/*z*/Fr	*P*
A1	61.5 (17)	>0.05	52.5 (15)	>0.05	72 (18)	>0.05	78 (18)	>0.05
A2	65.5 (16)	>0.05	71 (16)	>0.05	89 (17)	>0.05	68 (17)	>0.05
B1	0.602 (26)	>0.05	8.551 (26)	**<0.02**	10.431 (26)	**<0.01**	0.857 (21)	>0.05
B2	2.571 (21)	>0.05	15.311 (21)	**<0.01**	7.238 (21)	**<0.05**	0.857 (21)	>0.05
C1	60 (15)	>0.05	30 (14)	>0.05	70 (15)	>0.05	44.5 (15)	>0.05
C2	37 (13)	>0.05	13 (10)	>0.05	43 (13)	>0.05	26 (13)	>0.05
C3	−2.2732 (31)	**<0.01**	69 (25)	**<0.02**	−3.5078 (31)	**<0.001**	−1.8100 (30)	>0.05
C3	10 (11)	**<0.05**	0 (7)	**<0.02**	9 (11)	**<0.05**	22 (11)	>0.05
D	7 (10)	**<0.05**	4 (11)	**<0.01**	11 (11)	**<0.05**	27 (10)	>0.05

Sample size is shown in parentheses. The analyses were done by Wilcoxon matched-pairs signed-ranks tests (statistic: *T* and *z* for samples >25) except for B1 and B2, in which we used Friedman two-way analyses of variance (statistic: Fr). Significant differences in bold.

in test phases were not the effect of an inherent between-group difference in crab behavior.

In the test phase of experiment B1, β crabs investigated the target shell more often in the presence of odor from familiar conspecifics than with their own odor (Figure 2B, after multiple comparisons test: α crab odor = γ crab odor > self-odor). Shell investigation was also longer in the presence of α crab odor (Figure 2C, after multiple comparisons test: α odor > γ odor = self; Table 2). Different odors had no apparent effect on latency (Figure 2A) or on the duration of locomotion (Figure 2D). In experiment B2, β crabs discriminated between their own odor and the odor released by conspecifics, but they did not differentiate between unfamiliar α and γ crabs (Figure 2B, after multiple

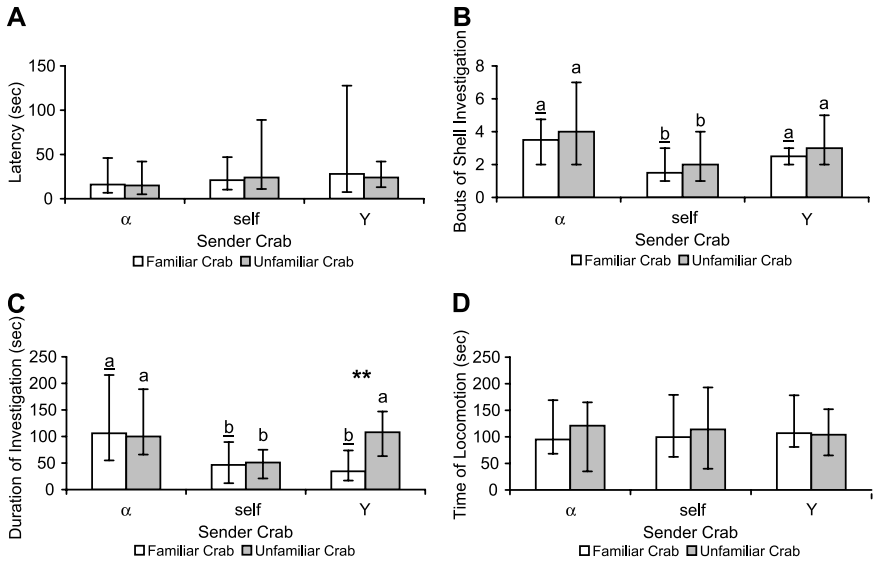

FIG. 2. Results from experiment B: discriminating odor between familiar/unfamiliar conspecifics with different attributes. Median values (and interquartile ranges) of latency (A), bouts of shell investigation (B), duration of shell investigation (C), and time of locomotion (D), compared between familiar (experiment B1) and unfamiliar (experiment B2) crabs, for three odor sources: α crabs, the test crabs (self, β crabs), and γ crabs. Alpha (and γ) crabs were the bigger (and smaller) crabs occupying a larger (and a smaller) shell compared to the β test crabs. See Table 2 for test statistics. Letters above bars (underlined for familiar crabs and not underlined for unfamiliar crabs) indicate differences among odor treatments (multiple comparisons test after Friedman two-way analysis of variance); ** denotes $P < 0.01$.

comparisons test: α odor = γ odor > self-odor; Figure 2C, after multiple comparisons test: α odor = γ odor > self-odor; Table 2).

Experiment C: Associating Odor of Familiar Conspecifics with One of Its Attributes. As in experiment B, trios used in C1, C2, and C3 showed the same agonistic level during the familiarization phase (number of fights: $H = 1.1614$, $df = 2$, $P > 0.05$; $H = 5.3524$, $df = 2$, $P > 0.05$; duration of fights: $H = 1.2573$, $df = 2$, $P > 0.05$; $H = 3.2290$, $df = 2$, $P > 0.05$), thus excluding inherent between-group differences in crab behavior.

In the test phase, crabs reacted in the same fashion to the target shell when the attributes of the senders differed for their rank only (experiment C1) or for their size (and rank) (experiment C2; Figure 3). However, in experiment C3, β

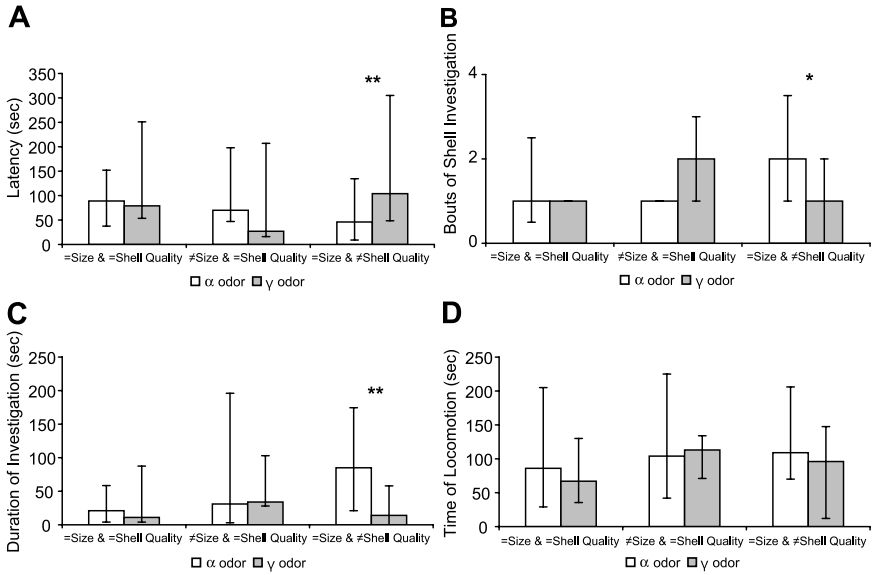

FIG. 3. Results from experiment C: associating odor of familiar conspecifics with one of its attributes. Median values (and interquartile ranges) of latency (A), bouts of shell investigation (B), duration of shell investigation (C), and time of locomotion (D), comparing treatments with odor from α and γ crabs in three conditions (same body size and same shell quality, C1, different body size and same shell quality, C2, and same body size and different shell quality, C3). Alpha (and γ) were the dominant (and subordinate) crabs in condition C1, the bigger (and the smaller) crabs in condition C2, and the crabs occupying a larger-than-optimal (and a smaller-than-optimal) shell in condition C3. See Table 2 for test statistics; * and ** denote $P < 0.05$ and $P < 0.01$, respectively.

crabs showed a quicker response to the target shell (Figure 3A), and they investigated it more often (Figure 3B) and for a longer time (Figure 3C) when the odor was emitted by crabs that occupied LTO (i.e., α crabs), rather than STO (i.e., γ crabs), shells (Table 2). No difference was found for the duration of locomotion (Figure 3D).

Experiment D: Plasticity of the Association Between Individual Odor and Shell Quality. Altering the quality of the shells occupied by α and γ crabs modified the behavior of β crabs. The quality of the shell, but not the identity of the sender crab inhabiting it, affected shell investigation by β crabs. Receivers (β crabs) reacted quicker (Figure 4A), with greater frequency (Figure 4B), and for a longer time (Figure 4C) in the presence of odor from senders in LTO shells regardless of their identity. The duration of locomotion (Figure 4D) did not differ significantly between experiments C3 and D (Table 2).

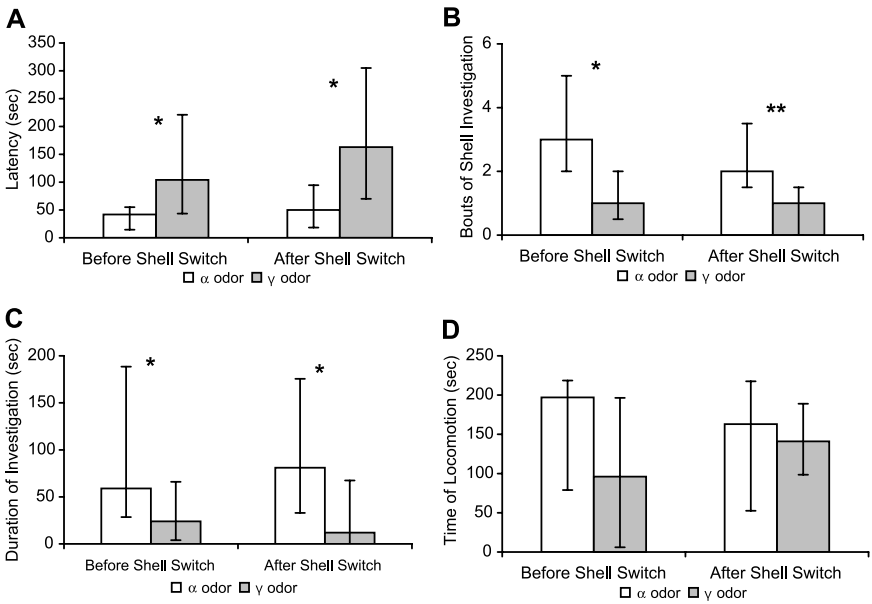

FIG. 4. Results from experiment D: plasticity of the association between individual odor and shell quality. Median values (and interquartile ranges) of latency (A), bouts of shell investigation (B), duration of shell investigation (C), and time of locomotion (D), comparing treatments with odor from α and γ crabs before (experiment C3) and after (experiment D) shell switch. Alpha (and γ) were the crabs occupying a larger-than-optimal (and a smaller-than-optimal) shell. Alpha (and γ) crabs in experiment D are the individuals that were ranked γ (and α) crabs in experiment C3. See Table 2 for test statistics; * and ** denote $P < 0.05$ and $P < 0.01$, respectively.

DISCUSSION

Our study revealed a number of properties that characterize the nature of individual recognition by odor in hermit crabs. First, it proved that *P. longicarpus* is unable to chemically recognize familiar and high-quality empty shells. Then, it showed that (1) there is no odor specific of a rank; (2) individual crabs discriminate their own odor from the odor of other individuals; (3) they can chemically discriminate between larger crabs inhabiting higher-quality shells and smaller crabs inhabiting lower-quality shells, provided that these crabs are familiar to them; (4) they associate the odor of an individual crab with the quality of the shell it inhabits; and (5) this association quickly changes when the social partners switch to shells of different qualities.

By excluding that individual recognition by hermit crabs could be simply a consequence of their ability to learn shell odors, this study confirms Gherardi and Tiedemann's (2004a) and Gherardi and Atema's (2005b) findings in the same species. To the contrary, Jackson and Elwood (1989) showed that *P. bernhardus* is able to discriminate, at least by sight, between familiar and novel empty shells, even if seemingly identical, by remembering certain subtle features of already investigated shells. Our results are also consistent in part with Gherardi and Tiedemann (2004b), who showed that *P. longicarpus* identifies its own odor from the odor of other individuals and is capable of chemically discriminating between familiar and unfamiliar conspecifics.

However, in Gherardi and Tiedemann's (2004b) study and in the majority of other studies on individual recognition in invertebrates (see Caldwell, 1985 for an exception), the tasks employed measured, at best, differences in responses to two "heterogeneous subgroups" of conspecifics (Barrows et al., 1975), i.e., familiar and unfamiliar subgroups. That is, results of such experiments simply arrived at documenting that the study animals were capable of a "binary" discrimination among opponents (Boal, 1996), but not of "true individual recognition" (i.e., the ability to discriminate one individual of a group from every other individual on the basis of "a unique set of cues defining that individual"; Beecher, 1989).

Our study has partly overcome this methodological limitation. By investigating graded animal responses to a target shell in the presence of odors of different provenience, we found that hermit crabs can chemically discriminate (1) between themselves and others and (2) between at least two familiar individuals with different attributes. As a consequence, *P. longicarpus* seems to rely on a form of recognition that is more complex than a simple binary system. Because crabs seemed not to discriminate among unfamiliar crabs with the same attribute differences as the familiar crabs, the ability to chemically recognize at least two different familiar individuals was not because of odors proper of a rank, of a size class, or of a shell type. To the contrary, our results

might be explained by hypothesizing that during the familiarization phase, the odor from a social partner is associated by the receiver with the rank, the size, or the shell quality of the sender (or with a combination of these three attributes).

Experiment C illustrated that *P. longicarpus* can associate odor from a conspecific with the quality of the shell it occupies and reacts accordingly in the presence of a target shell. It is likely that during the familiarization phase, individual odors became labels of shell quality; if these labels indicate a high shell quality, their detection evokes an intense shell investigation when the receivers are presented with a high-quality shell; otherwise, shell investigation would have been scarce or absent. This view was supported by experiment D that also showed the plasticity of individual odor–shell quality association. Once an individual crab had switched to a shell of a different quality, responses to the offered shell were consistent with the changed association. Shell investigation was strong in the presence of odor of former crab γ in a high-quality shell and weak in the presence of former crab α in a low-quality shell.

An explanation for these results might be provided by the model of classical conditioning. This has been advocated to describe the dynamics of odor learning involved in food location by honeybees (e.g., Menzel, 1999) and in host detection by parasitoid hymenopterans (e.g., Kaiser et al., 2003). In our case, hermit crabs may memorize a stimulus (conditioning stimulus, i.e., the odor of a social partner) when it is associated with an unconditioned stimulus (i.e., the high quality of the shell occupied by the sender). The conditioned stimulus then becomes predictive of the reward (i.e., the potential acquisition of a high-quality shell) and elicits a conditioned response identical to the response normally elicited by the unconditioned stimulus (unconditioned response, i.e., investigating the offered shell). However, a difficulty in applying this model to odor learning in hermit crabs arises when we examine the nature of the reward. Associative learning is usually highly sensitive to unrewarded presentations of the conditioned stimulus, and in our experiments, we tested only individuals that had not been successful in acquiring crab α shell. As a consequence, if associative learning is the mechanism underlying acquisition and retention of memory of individual odors in hermit crabs, the reward for these organisms should be regarded as neither immediate nor certain, but rather as prospective and likely.

The question remains why this hermit crab species relies on individual odors of conspecifics to identify the quality of a shell. The answer remains speculative and thus provisional without any systematic fieldwork. Previous studies (Rittschof, 1980a,b; Rittschof et al., 1992) have shown that *P. longicarpus*, and particularly individuals inhabiting badly fitting shells (e.g., Rittschof, 1980b; Gherardi and Atema, 2005b), are chemically attracted to gastropod predation sites by fluids from partly digested snail tissue. At these sites, the attracted crabs form temporary and relatively small aggregations (Scully, 1978),

in which they agonistically interact to establish dominance hierarchies (Winston and Jacobson, 1978). The dominant crab obtains the first opportunity to occupy the empty shell as it is released by the predator (McLean, 1975); afterwards, other individuals exchange shells down the hierarchy. As a consequence, these aggregations function as "shell markets" and benefit a large number of the predation site attendants (Rittschof et al., 1992). Having obtained a high-quality shell, a crab generally leaves the predation site (Rittschof, 1980a; Tricarico and Gherardi, unpublished data), thus subtracting the shell from the market. It would be advantageous for an individual to rapidly classify the quality of the shells inhabited by other attendants and to spend time combating or negotiating (Hazlett, 1978) for a "really good shell." Because of water turbidity typical of many salt marsh habitats, chemical cues signaling shell quality might provide more reliable information than visual stimuli emitted by the shell itself; on the other hand, the exclusive use of tactile information from the shell would require time and energy consumption in repeated investigatory acts. Previous studies have shown that *P. longicarpus* quickly learns the chemical identity of a social partner (Gherardi and Tiedemann, 2004b; Gherardi and Atema, 2005b), is inaccurate in discriminating shells by sight (Gherardi and Tiedemann, 2004a), and often switches shells without prior investigation (Scully, 1986). However, we cannot exclude that sight and touch might integrate olfaction and lead to the improved detectability and discriminability of signals (Gherardi and Tiedemann, 2004b).

In this scenario, the plastic nature of the association between the individual odor of a conspecific and the quality of its shell has a clear adaptive value. Obviously, any shell exchange breaks the link between a given hermit crab and a given shell. The aggregations of hermit crabs around gastropod predation sites are characterized by a cascade of shell switches as the effect of a vacancy chain process (Chase et al., 1988). Therefore, the plastic response to the cues associated with high-quality shells is a key factor to optimize shell acquisition and to reduce errors. Indeed, *P. longicarpus* learned the odor of a conspecific after less than 30-min exposure to a stimulus animal (Gherardi and Atema, 2005b).

A final intriguing result of our study is an outline of the kind of representation of social partners that hermit crabs may have. We found that the sender, labeled by its individual odor, was classified by the receiver in function of the quality of its shell; on the basis of this classification, the receiver seemed to modulate the intensity of its investigatory acts toward the offered shell. The mechanism needed for this kind of recognition is one that involves in the receiver the association of a type of information from the sender (e.g., chemical cues) with memories of past experiences with it (e.g., exploration of its shell; for a general discussion, see Johnston and Bullock, 2001). This representation could be thought of as hermit crabs having a "concept" of other individuals, and hints at potential for relatively high-order knowledge about conspecifics.

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