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Concentration-dependent parsimonious releaser roles of gregarious male pheromone of the desert locust, *Schistocerca gregaria*

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Abstract

The responses of (i) groups of crowd-reared mature males of desert locust, *Schistocerca gregaria* to a choice of two columns of air, one permeated with different concentrations of phenylacetonitrile (PAN), the major component of gregarious-phase male-produced pheromone, and the other untreated, and (ii) individual crowd-reared mature males of the insect to varying concentration gradients of PAN, were studied in two different types of arena. In the choice assay, locusts preferred to be within PAN-permeated air column at low relative doses of the pheromone, but away from PAN at high relative doses. In the second assay, individual locusts were arrested close to PAN source at low PAN concentration gradients, but away from the source at high concentration gradients. The results are consistent with two reported releaser functions of the adult male-released pheromone that are dependent on different sensory thresholds: arrestment and cohesion at lower relative concentrations and male–male homosexual avoidance at higher relative concentrations.

Keywords: Pheromone parsimony; Aggregation; Male–male avoidance; Desert locust; *Schistocerca gregaria*

1. Introduction

The mediation of olfactory signals in the cohesive behaviour of locusts was first recognised by Nolte (1963) and later confirmed by Gillett (1968) and Gillett et al. (1976) following observations that isolated individuals of several locust species, including the desert locust, when kept in the same room with crowded locusts, continued to retain grouping traits of the gregarious phase. Antennectomy of crowd-reared adults and nymphs of *Schistocerca gregaria* induced solitarisation (Mordue, 1977; Heifetz et al., 1996), consistent with the critical role of olfactory chemoreception in sustaining the gregarious phase of both stages of the insect (Hassanali et al., 2005). However, the specific role(s) played by olfactory signals in locust phase dynamics, as well as their chemical nature and sources, have been a subject of much confusion and some controversy (Gillett, 1983; Fuzeau-Braesch et al., 1988; Whitman, 1990; Obeng-Ofori et al., 1993; Hassanali and Torto, 1999; Seidelmann and Ferenz, 2002; Seidelman et al., 2005).

An early source of confusion relates to assumed primer role played by these signals in inducing phase shifts of solitarious locusts to the gregarious phase, hence the use of the term 'gregarisation pheromone' (Nolte, 1963; Nolte et al., 1970). Recent studies in different laboratories have shown that solitarious desert locust individuals are not affected in this respect by exposure to volatile emissions from their gregarious counterparts (Heifetz et al., 1996; Roessingh et al., 1998; Hassanali et al., 2005). In any case, solitarious stages do not emit behaviourally active volatile signals of the gregarious phase (Torto et al., 1994; Deng et al., 1996; Seidelmann et al., 2000), so it is unlikely that these would be involved in priming phase transformation of solitaria (Hassanali and Torto, 1999). Phase shifts result from actual physical contact between insects that experience crowding, and two stimuli have recently been identified as the principal mediating factors, a chemotactile stimulus associated with the hydrocarbon fraction of locust cuticle (Heifetz et al., 1996, 1997) and a site-specific

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mechanotactile stimulus associated with outer face of hind femurs (Hägele and Simpson, 2000; Roessingh et al., 1998; Simpson et al., 2001).

Another source of confusion relates to the chemical nature and origin of olfactory signal(s) resulting from phase shifts in the desert locust and the African migratory locust, *Locusta migratoria migratoriodes*. Nolte et al. (1970, 1973) traced the source of olfactory signal to hopper faeces from which 5-ethylguaiacol (locustol) was identified as the principal component of the 'gregarisation pheromone' of both *S. gregaria* and *L. m. migratoriodes*. Subsequent analyses in different laboratories failed to detect 5-ethylguaiacol in any airborne volatiles of locusts or their faeces (Fuzeau-Braesch et al., 1988; Francke and Schmidt, 1994; Obeng-Ofori et al., 1994 a, b; Torto et al., 1994, 1996). Crowd-reared nymphal and adult desert locusts were found to emit signals of different chemical compositions (Torto et al., 1994, 1996; Obeng-Ofori et al., 1994b; Hassanali and Torto, 1999). Both sexes of the gregarious nymphal stages (second to fifth instars) emit a blend of medium-chained aldehydes and acids (C_6–C_8), together with phenol, guaiacol, and indole (Torto et al., 1996; Hassanali and Torto, 1999). The benzene derivatives are also present in their faeces (Obeng-Ofori et al., 1994 b). Emission from adults occurs in immature (10–12 days after fledging) and mature males (>24 days after fledging) and is made up of a series of benzene derivatives of which phenylacetonitrile (PAN) represents the major component (~80%) (Torto et al., 1994; Deng et al., 1996). PAN is also present in relatively large proportions in adult faeces (Obeng-Ofori et al., 1994b).

The releaser function(s) of these volatile signals have been a subject of a recent controversy. Fuzeau-Braesch et al. (1988) had earlier found that specific constituents identified from volatiles trapped from air surrounding crowd-reared *S. gregaria* and *L. m. migratorioides* did not elicit anemotactic attractive responses—unlike their responses to wind-borne grass odours (Kennedy and Moorhouse, 1969)—but instead elicited a clumping behaviour from groups of the insects, and the volatiles were therefore considered to function as a cohesion pheromone. Such cohesive responses were later studied in detail in a single-chamber arena with two columns of air, one untreated and the other enriched with different volatiles emitted by gregarious nymphal or adult *S. gregaria*, or antennographically active constituents (individually or in blends) identified in these volatiles (Obeng-Ofori et al., 1993, 1994 a, b; Torto et al., 1994, 1996). The results showed that, whether released in groups (visual stimulus present) or individually (no visual stimulus), the insects made clear choices in favour of a relevant signal (e.g. groups of or individual adult locusts to male adult emission or to PAN). Moreover, there was an interesting stage differentiation in the responses of nymphal and adult stages indicating the operation of distinct cohesion pheromonal effects in the two stages consistent with their different chemical compositions (Hassanali et al., 2005). On the other hand, in a recent study with mature crowd-reared *S. gregaria*, Seidelmann and Ferenz (2002) observed a different effect of PAN. In mating experiments, crowd-reared males were found to make pairing attempts with or to jump on solitary-reared males, but not with crowd-reared males nor with crowd-reared male–female mating pairs. When solitary-reared males or crowd-reared females were treated with PAN, no pairing attempts by gregarious males were observed. The authors concluded that PAN acts as a repellent in the desert locust and functions solely as a courtship-inhibiting pheromone (Seidelmann and Ferenz, 2002; Ferenz and Seidelmann, 2003).

In a follow-up study, Seidelman et al. (2005) documented the responses of different stages (fifth-instar nymphs, young and mature male and female adults) of crowd-reared desert locust individuals released downstream in a Y-shaped olfactometer with an upstream choice of clean air in one arm and another treated with PAN with and without other constituents of the adult male pheromone blend. Within the observation time (180 s), only a small proportion of the insects (e.g. between 13.2% and 27.7% of the released mature males at different PAN doses) moved up the pheromone plume to the arm of the olfactometer that functioned as the pheromone source. A large proportion of the insects was located either close to the release point or further downstream near the exit of the olfactometer. The authors interpreted this distribution pattern as confirming the repellent action of PAN on the insect and downstream movement toward the exit as 'escape reaction'. However, the bioassay design was based on the assumption that any aggregative effect of the pheromone would be reflected in an anemotactic response analogous to previously observed upwind movement of the insects mediated by plant kairomones (Kennedy and Moorhouse, 1969). This is contrary to earlier observations made by Fuzeau-Braesch et al. (1988), who suggested that cohesion of the insects results not from anemotaxis but from its arrestant effect. The design of the olfactometer that we developed to study pheromone-mediated cohesive behaviour of desert locusts (Obeng-Ofori et al., 1993) was inspired by these insights, which were also confirmed by our own preliminary observations in a wind tunnel where the insects demonstrated no predisposition to move up pheromone plumes and appeared to be located wherever they had olfactory contact with the signal (Njagi et al., unreported observation). Interestingly, although the distribution pattern of individual adult locusts reported by Seidelman et al. (2005) is complicated by the asymmetrical design of the olfactometer, and modified somewhat by increasing PAN concentration, it is consistent with the insect being arrested wherever it could perceive the pheromone, including the plume exit.

In a recent review of the chemical ecology of locusts, we proposed that the two reported releaser functions of the male adult pheromone as reflected in its major constituent (PAN) need not be mutually exclusive, and that both were possible if homosexual avoidance between gregarious

males occurs at relatively high concentrations close to the sources, while a cohesive effect occurs at lower concentrations away from these sources (Hassanali et al., 2005). We tested the validity of such concentration effects by studying the responses of crowd-reared, mature males to increasing doses of PAN in two types of arena. Herein we report the results of our study.

2. Materials and methods

2.1. Insects

Crowd-reared mature (yellow) desert locust males were obtained from the ICIPE colony originating from stocks obtained from the Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Addis Ababa, Ethiopia and ICIPE Port Sudan Field Station, with regular inclusion of fresh collections from the field. Mixed sexes of the insect were reared under crowded conditions (50–100) in aluminium cages ($50 \times 50 \times 50\,cm^3$) in a well-aerated room ($4.5 \times 4.5\,m^2$) with a duct system (10–15 air changes/h) that maintained a negative pressure, temperature range between 30 and 35 °C and a light–dark cycle of 12:12 h (Ochieng-Odero et al., 1994).

2.2. Aggregation responses at different doses of PAN

These assays were conducted in a glass chamber ($60 \times 30 \times 30\,cm^3$) described previously (Obeng-Ofori et al., 1993) designed to provide a clear choice to locusts between spatially equivalent columns of air, one containing varying concentrations of PAN and the other clean to which they can choose to escape. Briefly, clean air from a compressed cylinder (British Oxygen Company Kenya Limited), further purified by passing through a charcoal filter was split into two streams, each passing through a separate 2-l round-bottomed flask and then distributed into one of the two sides of the arena at a flow rate of 120 ml/(min/side). One flask contained different doses of PAN (0, 2, 4, 8, 16, 32, 64, 80, 96 and 128 μl) in 2 ml of (Merck) paraffin oil (to moderate its release rate) in screw capped 3.7 ml glass vials with 1.5 mm vents in the caps (Torto et al., 1994). Each μl of PAN corresponded to ~12.5 h of emission from an average male gregarious locust from a fresh laboratory colony (Torto et al., 1994). Thus, the dose range used in the present study went beyond that used previously (<32 μl) in our studies (Torto et al., 1994). PAN was delivered from vials in dichloromethane (0.5 ml), which was allowed to evaporate off before addition of paraffin oil. The second (control) flask contained a vial with paraffin oil (2 ml). To ensure the presence of comparable level of residual dichloromethane, the same amount of the solvent (0.5 ml) was initially placed in this vial and allowed to evaporate off over the same period before addition of paraffin oil. Blank controls were initially run, and control and treatment flasks were switched regularly to either side of the chamber during tests to avoid any position bias. The olfactometer was located in an exclusive laboratory maintained at 30 ± 1 °C below an extraction hood (to avoid accumulation of PAN) and fitted with two fluorescent light tubes (60 cm, 60 W) to provide uniform lighting.

Tests were carried out on groups of six mature males, which were introduced into the arena through a door of a small introduction chamber located at the middle, bottom part of the arena (Obeng-Ofori et al., 1993). Every 15 min for 1 h, the number of insects on both sides of the arena was counted. All tests were replicated eight times, each involving fresh, previously unused insects. In some observation cycles, a few insects were found in the middle part of the arena facing different sides of the chamber and may or may not have had olfactory contact with the pheromone. These were not classified. For each replicate, the average retention (aggregation) index from four readings was calculated as $100 \times (T-C)/N$, where T is the number of locusts found in the treated section of the arena, C is the number in the control section, and N is the total number of insects introduced. The average retention indices from eight replicates were plotted as a function of PAN dose.

2.3. PAN gradient effects on locust position

A rectangular ($84 \times 7 \times 7\,cm^3$) glass chamber (Fig. 1) was constructed and it was designed to restrict locust movements essentially within a linear, tunnel-like space, in order to facilitate accurate location of positions preferred by individual locusts in response to different PAN gradients. The two ends of the chamber were fitted with 40-mesh nylon gauze (B) to avoid accumulation of PAN diffusing from the centre (D) and, thus, to facilitate formation of concentration gradients of the compound along the length of both sides of the chamber. A small opening ($12 \times 6\,cm^2$) on the top of the chamber (A) with a tightly fitting glass lid allowed the introduction of PAN source and a test locust at the centre of the arena. A rectangular nylon gauze (also 40-mesh) was glued to fit the glass floor (C) to facilitate easy movement of the test locust. The chamber was placed on top of a rectangular table with transluscent glass top. Two fluorescent light tubes (60 cm, 60 W) 55 cm below the table top along the

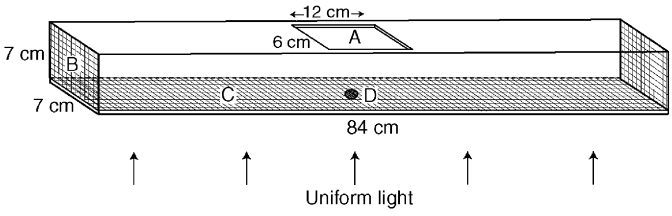

Fig. 1. Glass chamber to study the preferred position of individual mature males in relation to concentration gradients of PAN. A, window (with a glass lid) for introduction of test insect; B, nylon mesh barrier; C, nylon mesh flooring on glass bottom; D, 1 cm diameter filter paper (Whatman no. 1) with PAN in paraffin (5 μl) on a glass slide cover ($1\,cm^2$).

length of the chamber provided uniform illumination from below. The chamber was located in an exclusive laboratory with an extraction fan and maintained at 30±2 °C.

Various doses of PAN (0.125, 0.25, 0.5, 1.25, 2.5 and 5.0 μl) in paraffin oil (5 μl) were dispensed on a filter paper disc (1 cm diameter Whatman no. 1) placed on a glass slide cover (1 cm^2) at the centre of the chamber floor. After 5 min, a mature male locust was introduced and observed for 15 min. At the end of this period, the resting position relative to PAN source adopted by the locust (its face from the centre of the arena) was noted. The locust was then removed and another was introduced. After every three introductions of locusts, the arena was cleaned thoroughly and dried in the oven, and the observations repeated with fresh PAN dispensers and locusts. For every dose, 15 replicate observations with different locusts were made. The average locations of locusts at different PAN doses were compared to determine if there was a dose-dependent trend.

2.4. Analysis of data

The effects of dose on the aggregation index of locust or position assumed by individuals relative to PAN source were analysed by one-way ANOVA using PROC GLM of SAS (SAS Institute 2002). Means were separated by Student–Newmann–Keuls (SNK) test at 5%.

3. Results

3.1. Aggregation responses to different PAN doses

More than 90% of the insects were distributed in varying proportions between the treated or untreated side of the single chamber arena during different observation cycles. The average percentage (±SE) of groups of six mature male locusts that were located at the middle of the arena at different doses, and were thus not classified, were as follows: 2.6±1.1 (0 μl), 4.9±1.5 (2 μl), 5.2±2.8 (4 μl), 9.8±1.1 (8 μl), 6.8±1.9 (16 μl), 7.8±2.1 (32 μl), 9.4±3.6 (64 μl), 2.1±1.1 (80 μl), 3.1±1.0 (96 μl) and 4.2±1.5 (128 μl). Fig. 2 summarises the selection behaviour (±SE) of these insects to increased doses of PAN. The results show that a six-fold increase in the dose (from 16 to 96 μl) changed the preferred location of the locusts from PAN-permeated air column to the PAN-free side, indicating a shift from preference to avoidance behaviour.

3.2. Preferred position of individual locusts at different PAN gradients

Fig. 3 depicts the average location of individual locusts relative to different PAN doses at the centre of the rectangular arena. In the absence of PAN, each locust moved rapidly on either side of the chamber, and toward the end of 15 min it was located about 23.0±3.1 (SE) cm from the centre. The presence of low doses of (0.125 and 0.25 μl), however, retained the locust close to the pheromone. Higher doses of PAN resulted in movement away from the pheromone source in a dose-dependent fashion. Interestingly, the two highest doses used in the tests elicited immediate avoidance response from the exposed individuals, which moved close to their resting positions within the first 5 min.

4. Discussion

Results of this study show that *S. gregaria* males demonstrate concentration-dependent responses to PAN, the major component of their gregarious-phase pheromone emission. When exposed to varying concentration gradients of PAN in the rectangular arena, individuals were arrested closer to PAN source at low relative doses of the pheromone, but further away at higher relative doses (Fig. 3). This behaviour is reflected in bimodal responses of the insects between columns of clean air and air permeated with different concentrations of PAN (Fig. 2). In a gregarious desert locust population, since pheromone from each source diffuses in all directions, a steep concentration gradient can be expected around each male locust. A close encounter between two males would result in exposure of both to enhanced levels of the pheromone, which accounts

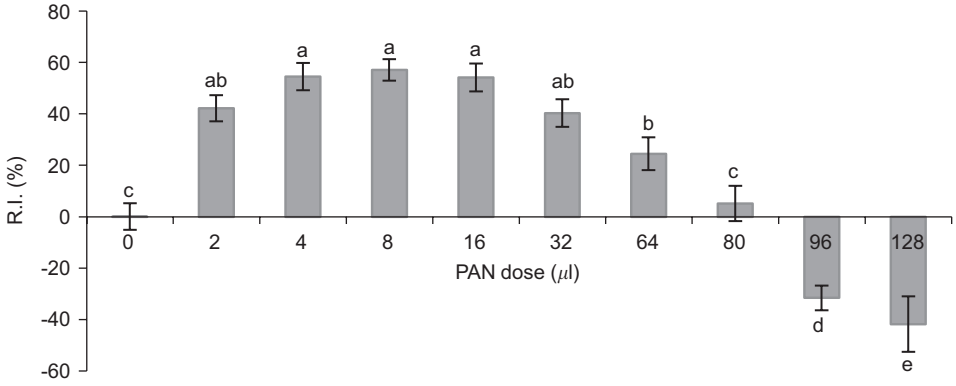

Fig. 2. Preference (aggregation) indices (±SE) of groups of mature male *S. gregaria* in a choice olfactometer at different doses of PAN. Means with different letters are significantly different ($P<0.05$; SNK test).

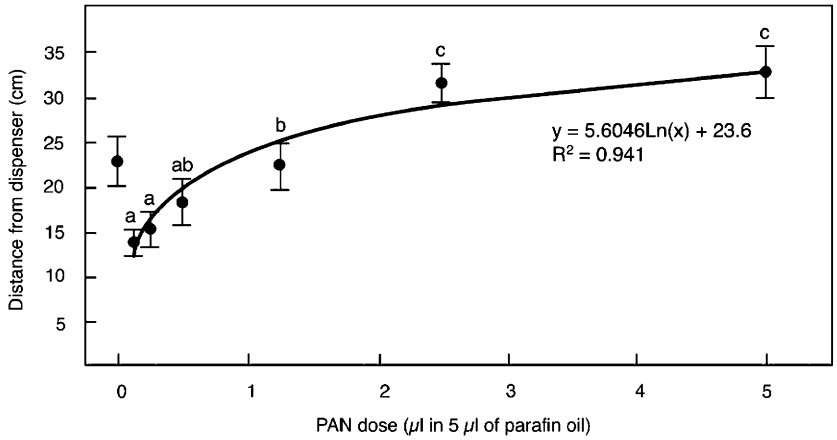

Fig. 3. Preferred location of single mature males *S. gregaria* (±SE) relative to different concentration gradients of PAN in a rectangular arena. Means with different letters are significantly different ($P<0.05$; SNK test).

for the homosexual avoidance demonstrated by Seidelmann and Ferenz (2002). However, further away from one another, a cohesive effect of the pheromone would operate as demonstrated previously in our laboratory (Obeng-Ofori et al., 1993, 1994a, b; Torto et al., 1994). Our results are then consistent with dual releaser functions of the adult male-released pheromone that are dependent on different sensory thresholds: arrestment and cohesion at lower relative concentrations and avoidance (repulsion) at higher relative concentrations.

Although recent data presented by Seidelman et al. (2005) are complicated by the asymmetrical design of the Y-shaped olfactometer, they show an interesting dose-dependent pattern. Interestingly, decreasing PAN concentrations evoked a greater tendency in individual crowd-reared males to be where the insect had olfactory contact with the chemical signal (near the introduction point, downstream at the plume exit or upstream in the arm functioning as PAN source), compared with where it had no contact with this signal (second arm of the olfactometer). Indeed, the percentage of the insects that chose the arm without PAN, decreased from 44.6% at the highest dose used (87.6 µg/l) to 23.4% at the lowest dose (43.1 ng/l). However, the authors interpreted this trend as an 'arousal' effect of lower doses of PAN rather than the 'arrestment' of the insect by this chemical signal. If PAN is indeed a repellent at all dose ranges (as implied by these authors), how do locusts stay cohesive? Seidelman et al. (2005) recognised the problem and speculated that aggregation may arise from the 'arousal' effect of low doses of PAN, or the rest of the male-produced pheromone blend, signalling the presence of other gregarious individuals and inducing visual attraction between them. However, it is difficult to see how the balance of such opposing effects (repulsion and attraction) could lead to the effective cohesion that is characteristic of gregarious locusts. On the other hand, the results of our present study confirm that a lower dose range of PAN (2–32 mg, corresponding to 25–400 locust hours), comparable to that used in our previous studies (Torto et al., 1994), elicits responses (however characterised: 'arousal' or 'arrestment') in individuals that lead to the congregation of locust groups. This is consistent with our earlier finding that, whether released individually (no visual cue) or in groups (visual cues present) in the single-chamber arena, locusts preferred to be within the precinct of the air-column enriched with natural pheromone emissions from live insects (Obeng-Ofori et al., 1993), indicating that pheromonal communication may be the principal mechanism modulating the aggregation behaviour of the gregarious-phase desert locust.

Unlike their adult male counterparts, female locusts do not emit the aggregation pheromone. In our previous studies, adult females were found to respond to the male-produced pheromone in the presence (Obeng-Ofori et al., 1993; Torto et al., 1994) or absence (Obeng-Ofori et al., 1994a) of conspecific males, which is consistent with their active participation in adult swarms. Although mature females encounter above-average concentrations of the pheromone during mating interactions with males, this represents a significantly lower concentration of the chemical signal compared to the combined emissions from two males in homosexual encounters. However, it would be interesting to see how females respond to similar elevated concentrations of the pheromone.

The two releaser functions of the male-emitted adult pheromone represent part of multifunctional role of this chemical signal that includes its primer effects. One of the earliest examples of primer pheromonal activity reported for any insect was the maturation accelerating effect of volatile emissions from gregarious-phase mature male desert locusts on young males and females (Norris, 1954, 1964; Norris and Pener, 1965; Richards and El Mangoury, 1968). Recent studies with synthetic constituents of the pheromone, including PAN, on groups of young adult desert locusts have confirmed these accelerating effects (Mahamat et al., 1993, 2000). Interestingly, the nymphal pheromone blend responsible for cohesive behaviour of the nymphal stages retards the maturation of young adults and

it has been proposed that sequential exposure of early and late fledgers to inhibitory and acceleratory signals, respectively, contributes to the development of maturation synchrony in gregarious locust populations (Norris, 1964; Richards and El Mangoury, 1968; Assad et al., 1997; Hassanali et al., 2005). Another primer effect of the adult pheromone (and also of nymphal pheromone) relates to the earlier observations that isolated locust individuals kept in the same room as crowd-reared counterpart continue to retain the pigmentary, morphometric, and cohesive traits of gregarious conspecifics (Nolte, 1963; Gillett, 1983). Although these findings were misinterpreted to signify the ability of the volatile pheromonal signal to induce phase shifts in solitaria to the gregarious phase, the observations indicated that gregarious individuals that were isolated from parent colonies but continued to be exposed to their pheromone emissions did not solitarise as rapidly as isolated individuals that were not similarly exposed. This has been confirmed in a recent study where isolated crowd-reared nymphs and adults of the desert locust that had olfactory contact with pheromone emissions of gregarious groups solitarised significantly more slowly than their counterparts that were denied such contact (Hassanali et al., 2005). Adaptation of pheromones (and other semiochemicals) to serve multiple functions in different contexts (described as 'pheromone or semiochemical parsimony') has been observed in many arthropods, but particularly in eusocial insect societies (Blum, 1996). Gregarious-phase desert locusts demonstrate a relatively complex social structure almost comparable to those of social insects, and the different releaser and primer functions of the gregarious male-produced pheromone demonstrate an interesting case of evolutionary response of the species to the different demands of a cohesive population.

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