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Effects of sensory stimuli on the behavioural phase state of the desert locust, *Schistocerca gregaria*

Peter Roessingh [a], Abdelghani Bouaïchi [b], Stephen J. Simpson [c,*]

[a] *Institute for Systematics and Population Biology, University of Amsterdam, Kruislaan 320 1098 SM Amsterdam, The Netherlands*
[b] *Centre National de Lutte Antiacridienne, B.P. 125, Inezgane, Agadir, Morocco*
[c] *University of Oxford, Zoology Department and the University Museum of Natural History, South Parks Road, Oxford OX1 3PS, UK*

Abstract

The nature of stimuli, emanating from other locusts, which are effective in inducing gregarization in the desert locust was investigated. Isolated-reared fifth-instar nymphs were subjected to tactile, visual and olfactory stimuli, presented singly and in combination, and the effect on the behavioural phase state was quantified using logistic regression analysis. Tactile stimulation provided by rolling paper spheres proved to be highly gregarizing, whether presented alone or in combination with the other stimuli. Olfactory and visual stimuli together caused partial behavioural gregarization. Visual stimulation alone was weakly gregarizing after prolonged exposure, while olfactory stimuli alone were ineffective. Nymphs and pre-reproductive and reproductive adults of both sexes were also treated with synthetic adult male 'aggregation' pheromone blend (Torto et al., 1994, Journal of Chemical Ecology 20, 1749). No effect of this blend was found on the behavioural phase state, even when visual stimuli were present. Non-locust related stimuli, including wheat odour and flashing lights, were also tested on nymphs. Neither induced any change in the behavioural phase state, indicating that increased sensory flow is not a sufficient explanation for locust-induced behavioural phase change. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Behavioural phase transition; Olfaction; Aggregation pheromone

1. Introduction

Polymorphism (phase change) in response to variation in population density is a defining characteristic of the group of acridid grasshoppers called locusts (Uvarov, 1966). A key feature of locusts in the gregarious phase is their propensity to group together to form bands or swarms, whereas solitarious locusts tend to avoid one another (Kennedy, 1939; Ellis and Ashall, 1957; Roessingh et al., 1993). Behavioural changes in the desert locust *Schistocerca gregaria* can be observed after only a few hours of exposure to a group (Ellis, 1963; Roessingh and Simpson, 1994; Bouaïchi et al., 1995, 1996; Heifetz et al., 1996). Since the phase transition is density dependent, the altered behaviour will generate a positive feedback loop that, in combination with favourable environmental micro-structure (Roffey and Popov, 1968; Bouaïchi et al., 1996), pushes the population towards the gregarious phase.

While the density-dependent nature of the phase change is well established, there is considerable confusion in the literature about the nature of the stimuli provided by other locusts that induce phase change. Four types of stimuli from a group of locusts can be distinguished: visual, auditory, chemical and tactile. In many experiments, however, these stimulus sources were not independently evaluated. Work often quoted to prove the importance of visual stimuli produced by other locusts, (e.g. Ellis, 1959) does not fully separate the effects of visual and olfactory stimuli. The relative effect and influence on the phase state of sensory stimuli is therefore still largely obscure.

Locust phase is a complex phenomenon, influencing many aspects of the biology of locusts (Pener, 1991). It is well established that the various phase characteristics are affected with different timing to changes in density (Gillett, 1978 and references therein; Islam et al., 1994; Deng et al., 1995). Such differences can easily affect the

* Corresponding author.

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conclusions drawn from experimental manipulations and complicate comparisons between experiments. In addition, assays often focus on single aspects of phase. The work on 'aggregation' pheromones (Obeng-Ofori et al., 1993) for instance, is based on an assay that measures arrestment in response to olfactory stimuli emanating from gregarious locusts. Obeng-Ofori et al. (1994a) rightly stress that this response need not imply that effective stimuli directly influence phase state. Recently, Heifetz et al. (1996) used one of the active compounds from natural locust odour and suggested that it seems not to influence phase directly (it did, however, have an aggregating effect). These authors also suggested that chemotactile rather than olfactory cues mediated gregarization. Since synergistic effects between different compounds are often observed it is important to evaluate the effects of the full aggregation pheromone in an assay that directly measures the phase state of individual locusts.

Since behaviour is the first phase characteristic to change, and the determinant of further gregarization, we have developed a method that quantifies the behavioural phase state (Roessingh et al., 1993). Observed behavioural elements are integrated in a multidimensional model using logistic regression. A probability for being in the solitarious phase can be assigned to individual insects using the model, and this value can subsequently be used in an analysis of variance to assess the effects of applied treatments.

In the present paper we investigate the effect on the behavioural phase state of individual locust being subjected to locust-related sensory stimuli (tactile stimuli from paper balls, visual stimuli from other locusts, natural locust odours and synthetic adult male aggregation pheromone blend) as well as the response to some non-locust stimuli (flashes of light and the odour of fresh cut seedling wheat). Auditory stimuli appear to be unimportant (cf. Gillett and Phillips, 1977) and were not investigated.

2. Materials and methods

2.1. Insects

Fifth-instar nymphs (3–5 days after ecdysis), young adults (4–10 days after ecdysis) and mature adults (15–30 days after ecdysis) of both sexes were used. All locusts were reared for at least two generations in isolation (see Roessingh et al., 1993 for details). All experimental insects had access to food ad libitum up to the time of testing. They were each behaviourally assayed once and then discarded. Untreated locusts from two extreme forms (three- or four-generation isolated-reared and crowd-reared adults) were independently assayed and used to construct the logistic regression models. In addition, untreated crowd- and isolated-reared controls were tested periodically throughout the experiments.

2.2. Experimental designs

Four experiments were conducted.

1. An investigation of the single and interactive effects of visual stimuli (sight of 10 locusts), natural olfactory stimuli (air passed over 50 locusts) and tactile stimuli (10 moving paper balls) on fifth-instar nymphs. All eight possible stimulus combinations were used in a factorial design.
2. A study of the effects and interaction of a visual stimulus (visual stimulus = 10 locusts) and synthetic adult male aggregation pheromone blend (50 LH aggregation pheromone) on fifth-instar nymphs [1 LH = volatiles emitted by one locust in 1 h (Torto et al., 1994)]. All four possible stimulus combinations were provided in a factorial design.
3. The same stimuli and design as in experiment 2, but testing pre-reproductive and reproductive adult locusts.
4. A study on nymphs of the effects and interaction of food odours and flashing light, again with all four possible stimulus combinations provided in a factorial design.

Assays of the effects of treatments on locust behaviour were carried out blind throughout the experiments. Test insects, both nymphs and adults, were exposed to 4 h of one of the treatment combinations, except in experiment 2 where both 4 and 24 h treatments were used.

Throughout the experiments stimuli were applied separately or in combination using sets of two plastic boxes, a small inner chamber (13.5 cm long × 7.5 cm wide × 5.5 cm high) containing the solitary reared test insect, placed within a larger outer box (25.5 cm long × 14 cm wide × 9.5 cm high). The visual stimulus was provided by placing in the outer chamber 10 crowd-reared locusts of the same age and sex as the solitary reared test locust. The inner chamber was ventilated by passing a constant stream of charcoal filtered air (50 ml min^{-1}) into and out of the chamber through plastic tubing. In the treatments with natural olfactory stimuli the air stream was first passed through a 5 l conical glass flask containing cut seedling wheat or 50 crowd-reared locusts of the same age and sex as the test insect. For the aggregation pheromone treatments a source of about 50 LH (Torto et al., 1994) was placed in the inner chamber (see below). Non-specific visual stimulation was produced by enclosing treatment chambers in a large cardboard box, internally illuminated with a 40 watt bulb providing 200 lux. The bulb produced an irregular flashing pattern (generated with a fluorescent tube starter connected in series with the bulb) of about 2–5 Hz for 1.5 s. These flashes were alternated with 1 s of total darkness. Forced air cooling

was used to maintain room temperature (30°C) inside the cardboard box.

Tactile stimulation was provided by the inclusion in the inner chamber of 10 spherical papier maché balls (0.25 g, 1 cm diameter) and one glass marble for extra mass (4.5 g) The whole set-up was gently rocked ca. 5° from the horizontal with an electric motor and eccentric to keep the balls moving, providing irregular but persistent tactile stimuli to the treated insect.

2.3. Preparation of the pheromone blend

Of six electrophysiologically active aromatic compounds (Torto et al., 1994; Hansson et al., 1996) found in the volatiles of mature adult male desert locusts, four elicited the same behavioural effect as the crude volatile extracts. These are: phenylacetonitrile (the dominant component), benzaldehyde, guaiacol and phenol. A synthetic blend of the four compounds was found to be strongly active (Torto et al., 1994), inducing young and mature adults, but not nymphs, to remain within a vertical, odour-bearing airstream. The blend used in the present experiment consisted of phenylacetonitrile (80%), benzaldehyde (10%), guaiacol (5%) and phenol (5%); all obtained from Aldrich Ltd. Then, 1980 μl of paraffin oil, spectroscopic grade (Uvasol, Merck Ltd) was transferred into a 20 ml glass vial to which 1 μl of phenol was added. The mixture was warmed in a water bath for a few seconds to dissolve the phenol in the paraffin. Then 16 μl of phenylacetonitrile, 2 μl of benzaldehyde and 1 μl of guaiacol were added sequentially with shaking in-between additions. A stock solution of 10,000 μg of pheromone blend per ml was prepared; 200 μl aliquots of stock solution were transferred to 2 ml glass vials (ICIPE supplied, via Jencons Scientific Ltd), each containing 1800 μl of light paraffin oil. All vials were capped with plastic snap caps, with eight, 2 mm vents. A vial was fixed in the centre of each treatment chamber and left for 4 days at the bioassay temperature (30 ± 2°C) to allow the release rate to stabilize. The dispensers were weighed before and after exposure and the release rate was estimated to be 2.653 mg h^{-1}. This dose was equivalent to about 50 LH. The dispensers were constructed in co-operation with ICIPE and were prepared in exactly the same way as in the experiments of Torto et al. (1994).

2.4. Statistics

The probabilities for individual nymphs of being in the solitarious phase, called *p*(isolated), were determined using logistic regression. Details of this method can be found in Hosmer and Lemmeshow (1989) and Roessingh et al. (1993). Briefly stated, two groups of locust of known phase (crowd-reared or reared in isolation for three generations), were observed and subsequently used to build a model that, based on observed behavioural elements, calculates *p*(isolated) for locusts with an unknown behavioural phase state. For nymphs, the model from Roessingh et al. (1993) was used as well as a model based on 120 control insects observed during the experimental series (Table 1a). In the later model, 98.3 and 96.6% of the isolated- and crowd-reared nymphs, respectively, were correctly classified, thus giving an overall classification of 97.5% to their correct categories. The model χ^2 (testing the null hypothesis that all coefficients in the model are equal to zero) was 155.59 ($P < 0.001$ at 4 df). After removal of two outlyers, the model had a Pearson's χ^2 for goodness-of-fit (testing the null hypothesis that the observed probabilities do not differ from those predicted by the model) of 6.49 ($P > 0.999$ at 113 df). Seven behavioural variables were used to construct the model, four of these [final distance from the centre of the arena (called *x*-distance), walking and grooming frequencies and climbing time fraction], were retained in the model as significant indicators of phase. The climbing time fraction is the time the insect spends on the partition at the end of the arena, divided by the total observation time; see Roessingh et al. (1993) for a description of the other behavioural elements.

For adults, the logistic regression model was derived from 80 four-generation isolated-reared, immature and

Table 1
Behavioural elements and patterns retained in logistic regression models

a. Elements retained in model derived from untreated isolated-reared and crowd-reared nymphs

Variables in the equation	Coefficient β	Significance of change in the log likelihood ratio	Partial correlation coefficient
X-distance	− 5.8	< 0.001	− 0.01
Climbing time fraction	− 41.4	< 0.001	− 0.08
Grooming frequency	− 16022.3	< 0.001	− 0.05
Walking frequency	− 842.0	< 0.001	− 0.07
Constant	36.4		

b. Parameters retained in model derived from pre- and reproductive adults of both sexes (untreated isolated-reared and crowd-reared adults)

Variables in the equation	Coefficient β	Significance of change in the log likelihood ratio	Partial correlation coefficient
Climbing time fraction	− 8.3	< 0.001	− 0.16
Grooming frequency	− 2305.4	< 0.001	− 0.15
Walking frequency	− 530.3	< 0.001	− 0.21
Constant	9.6		

mature adults of both sexes versus 80 crowd-reared immature and mature adults of both sexes (Table 1b). After removal of two outlyers, the model categorized 96.8% of cases correctly (97.4% of isolated-reared and 96.3% of crowd-reared adults) and had a model χ^2 of 195.9 ($P < 0.0001$ at 3 df) with a value for Pearson's χ^2 statistic of 31.2 ($P = 1.0$ at 154 df). The behavioural elements retained in the model (climbing time fraction, grooming and walking frequencies) matched well with those reported earlier for adults (Bouaïchi et al., 1995).

Values of *p*(isolated) for individual experimental insects were rank transformed and normalized. The effects of the different treatments on the behavioural phase state were determined with analysis of variance performed in SPSS on these transformed data (Conover and Iman, 1981). When significant differences were found, the individual treatments were compared with the modified LSD procedure. This test corrects for the fact that multiple comparisons are made.

3. Results

3.1. Responses of nymphs to visual stimuli from other locusts, odour from other nymphs and tactile stimulation by paper balls

Values for *p*(isolated) for 111 experimental nymphs were computed using the model from Roessingh et al. (1993). The mean values and frequency histograms of *p*(isolated) of nymphs exposed to combinations of visual, olfactory and tactile stimulation are shown in Figs 1 and 2. Results from an analysis of variance on these data are presented in Table 2. A strong effect of tactile stimulation was observed. Visual and olfactory stimuli alone did not significantly alter behaviour, but the combination of the two stimuli caused a significant shift towards more gregarious behaviour in treated insects, indicated by a highly significant interaction between the two factors. The latter effect resulted in significant main effects for visual and olfactory stimulation, despite either alone being ineffective (see Fig. 1).

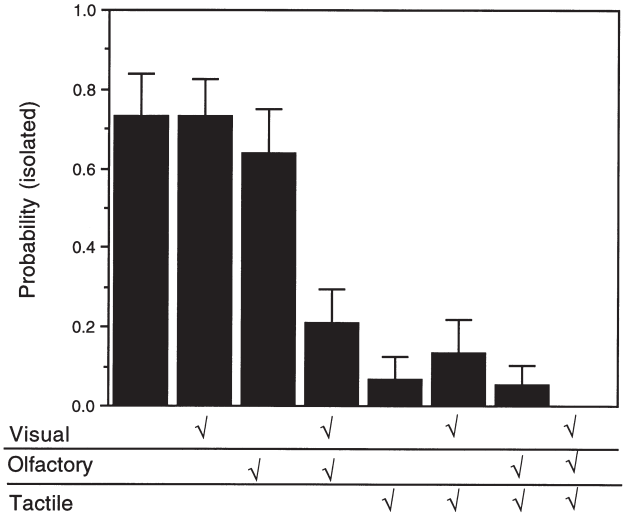

Fig. 1. Mean values (± S.E.) of *p*(isolated) for 111 experimental fifth-instar nymphs, each treated with one of eight combinations of visual stimuli (10 fifth-instar crowded-reared nymphs), olfactory stimuli (natural locust odour from 50 fifth-instar crowded-reared nymphs) and tactile stimuli (moving paper balls).

3.2. Responses of nymphs to visual stimuli and/or synthetic adult male aggregation pheromone blend

Forty nymphs were treated with visual stimuli and/or synthetic adult male aggregation pheromone blend. Values for *p*(isolated) were computed using the model from Table 1a. An independent set of 40 untreated, isolated-reared and 40 crowd-reared nymphs was also assayed and used for comparison. The mean values and frequency histograms of *p*(isolated) are shown in Figs 3 and 4. Results from the analyses of variance are presented in Table 3.

The synthetic pheromone blend produced no evidence of behavioural change when nymphs were treated in the absence of visual stimuli for 4 or 24 h. In contrast to the results in experiment 1, visual stimulation alone for 4 h did have a small but significant effect on the behavioural response of individual nymphs ($P < 0.001$ for comparison between the behavioural state after exposure and that of untreated, isolated-reared nymphs). A multiple comparison test revealed, however, that isolated-reared nymphs, given 4 h visual contact with crowd-reared insects, did not attain the level of the crowd-reared insects ($P < 0.01$). Subjecting isolated-reared nymphs for 24 h to visual stimulation (alone or in association with the synthetic pheromone blend) caused a much larger shift in the direction of the fully crowd-reared nymphs, and for this treatment there was no significant difference between the treated nymphs and crowded controls. It can be concluded that the adult aggregation pheromone blend alone did not affect the behavioural phase state of nymphs, while exposure to visual stimuli from other locust had a significant gregarizing effect, especially with longer exposure periods. Exposure period has a significant effect. No significant interaction between visual stimuli and synthetic pheromone blend was found.

3.3. Responses of adults to visual stimuli and/or synthetic adult male aggregation pheromone blend

Figs 5 and 6 show the behavioural responses of 160 pre-reproductive and reproductive male and female iso-

Fig. 2. Frequency histograms of the values of *p*(isolated) for 111 fifth-instar nymphs after treatment with one of eight combinations of visual, olfactory and tactile stimuli.

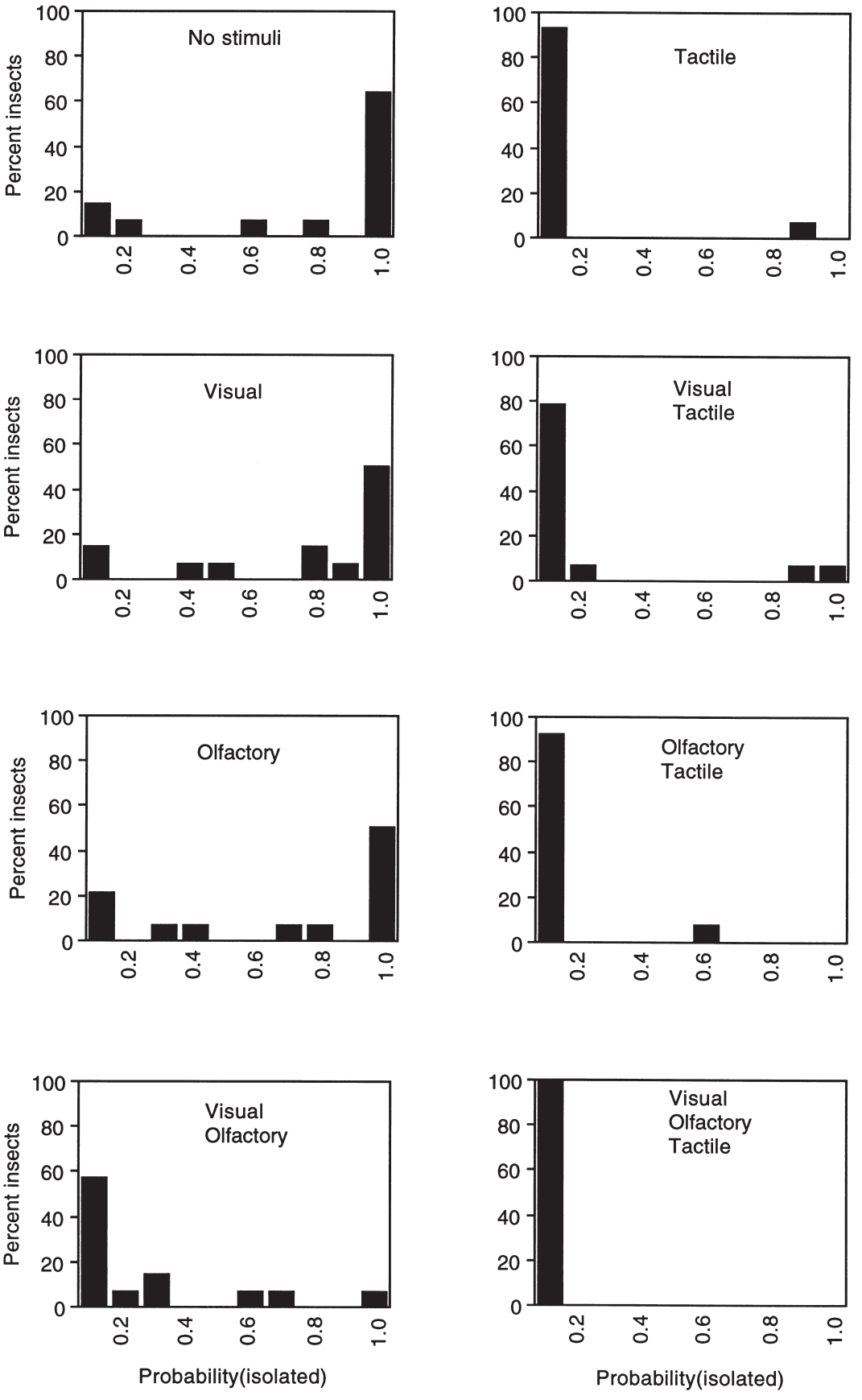
No stimuli
Tactile
Visual
Visual Tactile
Olfactory
Olfactory Tactile
Visual Olfactory
Visual Olfactory Tactile
Percent insects
Probability(isolated)
0
20
40
60
80
100
0.2
0.4
0.6
0.8
1.0

Table 2
Analysis of variance for normal scores from rank-transformed values of *p*(isolated) calculated from the model of Roessingh et al. (1993)

Source of variation	df	*F*	*P*
Main effects:			
Olfactory stimuli	1	12.88	0.001
Visual stimuli	1	7.20	0.008
Tactile stimuli	**1**	**112.41**	**< 0.001**
Interactions:			
Olfactory × Visual	**1**	**11.15**	**0.001**
Olfactory × Tactile	1	0.29	0.590
Visual × Tactile	1	0.18	0.671
Olfactory × Visual × Tactile	1	0.55	0.460
Residual	103		

Here, 111 fifth-instar isolated-reared nymphs were exposed for 4 h to olfactory stimuli (natural locust odour from 50 fifth-instar nymphs), visual stimuli (10 fifth-instar nymphs), and tactile stimuli (moving paper balls) in a factorial design and their behaviour was quantified in the bioassay arena. The relevant significant differences are marked in bold.

lated-reared adults to visual stimuli and/or the synthetic pheromone blend. Results from analysis of variance are presented in Table 4. No significant difference was found either between the behaviour of isolated-reared adults of different age and sex included in the model and those used as controls, or between the crowd-reared adults of different age and sex.

Subjecting adults to visual stimulation for 4 h, with or without synthetic pheromone blend, caused them to behave more gregariously than untreated isolated-reared controls ($P < 0.001$), but not at the level of untreated crowd-reared controls ($P < 0.001$). The synthetic pheromone blend presented alone induced no behavioural gregarization. No significant effect of age or sex was found, although there was an overall trend for immature adults to behave more gregariously than mature ones. The interaction term between visual and olfactory stimuli was not found to be significant, nor were the 3- or 4-way interactions.

3.4. Effects of light flashes and odour of seedling wheat

Values of *p*(isolated) for 76 nymphs (four times 19 insects, covering all four possible treatment combinations) were determined. Control insects had a mean value for *p*(isolated) of 0.85. Food odour and flashing light did not affect behaviour, yielding *p*(isolated) values of 0.88 and 0.81, respectively. The combination of the two stimuli did not affect the behavioural phase state [*p*(isolated) = 0.78], neither was there a significant interaction between these stimuli. *F* values were $F = 2.02$, $p = 0.16$ at 1 df; $F = 1.71$, $p = 0.20$ at 1 df; $F = 0.05$, $p = 0.83$ at 1 df, for food odour, flashing light and the combination, respectively.

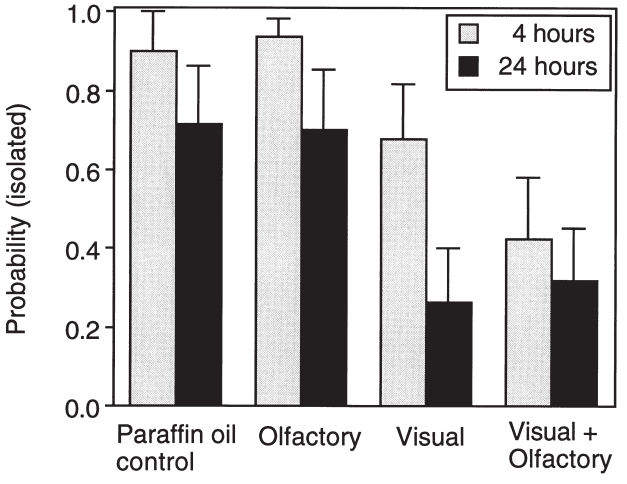

Fig. 3. Mean values (± S.E.) of *p*(isolated) for 40 experimental fifth-instar nymphs, treated with visual stimuli from 10 fifth-instar crowed reared nymphs and/or synthetic adult male aggregation pheromone blend.

4. Discussion

Our results indicate that tactile stimulation caused the most pronounced behavioural phase change. This is in agreement with reports in the literature (Chauvin, 1941; Ellis, 1959; Haskell, 1962). We found that visual and olfactory stimuli originating from other locusts are only weakly active as gregarizing stimuli when presented alone. However, a combination of visual and olfactory stimuli emanating from other locusts, or prolonged visual exposure (24 h), had a pronounced effect on the behavioural phase state of both nymphs and adults. These results show that care should be taken when comparing experimental results, since differences in

Fig. 4. Frequency histograms of the values of *p*(isolated) for 40 fifth-instar nymphs after treatment with visual stimuli from 10 fifth-instar locusts and synthetic adult male aggregation pheromone blend.

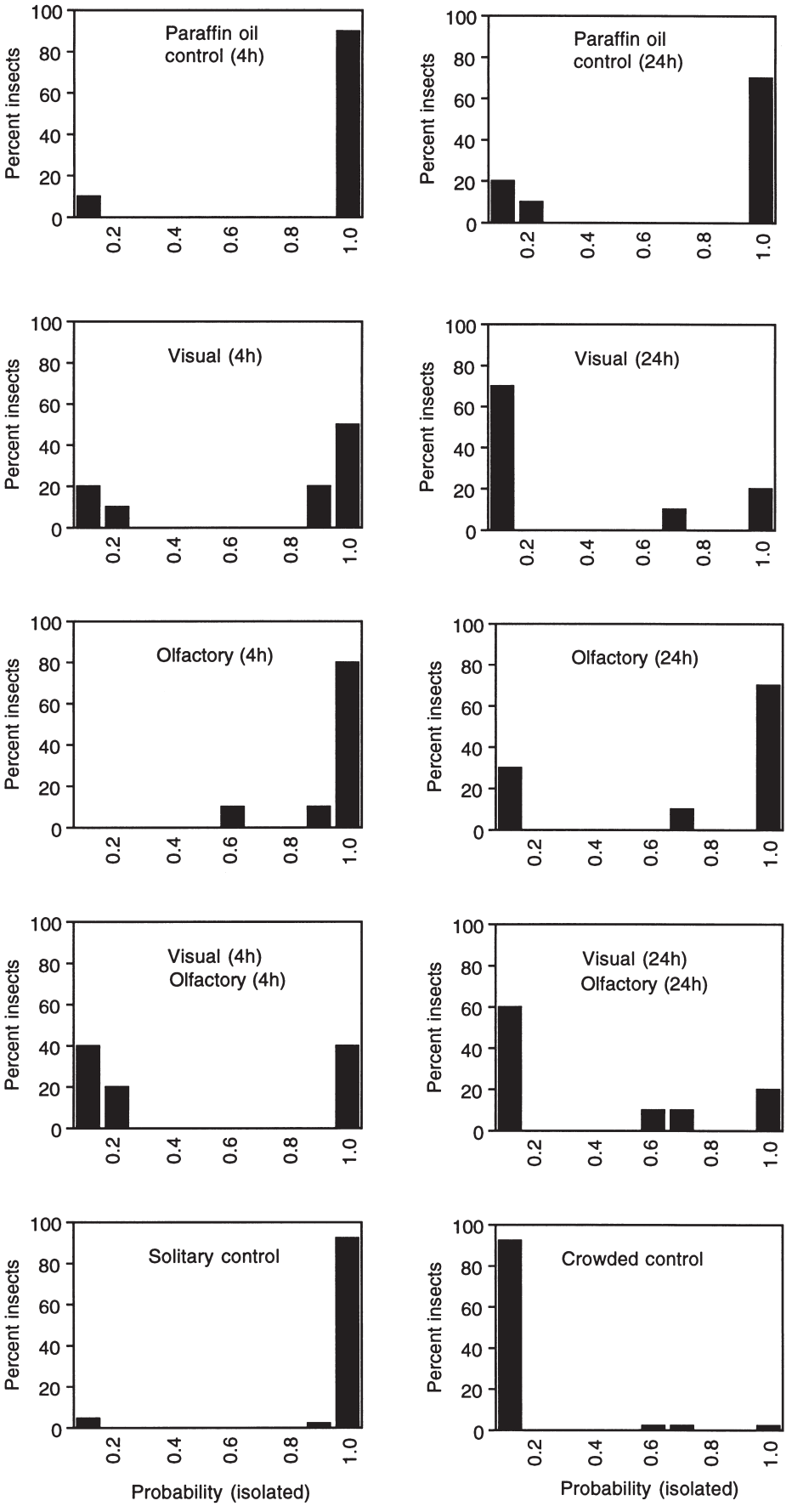
Paraffin oil control (4h)
Paraffin oil control (24h)
Visual (4h)
Visual (24h)
Olfactory (4h)
Olfactory (24h)
Visual (4h) Olfactory (4h)
Visual (24h) Olfactory (24h)
Solitary control
Crowded control
Percent insects
Probability (isolated)
0
20
40
60
80
100
0.2
0.4
0.6
0.8
1.0

Table 3
Analysis of variance for normal scores from rank-transformed values of *p*(isolated) calculated from the model in Table 1a

Source of variation	df	*F*	*P*
Main effects:			
Olfactory stimuli	1	0.67	0.415
Visual stimuli	**1**	**18.04**	**< 0.001**
Exposure period	**1**	**4.97**	**0.029**
Interactions:			
Olfactory × Visual	1	0.02	0.884
Olfactory × Exposure period	1	0.09	0.771
Visual × Exposure period	1	2.53	0.116
Olfactory × Visual × Exposure period	1	1.99	0.162
Residual	72		

Three-generation isolated-reared fifth-instar nymphs were exposed to visual stimuli (10 fifth-instar nymphs) and/or olfactory stimuli (synthetic adult male aggregation pheromone blend) and their behaviour was quantified in the bioassay arena, after either 4 or 24 h treatment.

exposure time might strongly influence the outcome of the experiments. While the relative lack of effect of visual stimuli alone was unexpected given the seeming consensus in the literature about their importance, close inspection of the primary literature show that very little evidence actually exists that unequivocally demonstrates a strong gregarizing effect of pure visual stimuli. Most references stem directly or indirectly from Ellis (1959) and Ellis and Pearce (1962), but these authors did not fully separate locust-derived visual and olfactory stimuli. Moreover, Heifetz et al. (1996) recently separated visual and olfactory stimuli (phenol derivatives, feacal extracts and trapped airborn volatiles from locusts) and concluded that visual stimuli alone did not change phase-related behaviour in their assay. Visual stimuli do, however, play an important role in the attraction of gregarious locusts to each other over short distances and in the mutual repulsion of solitarious locusts (Roessingh et al., 1993; Roessingh and Simpson, 1994; Islam et al., 1994; Bouaïchi et al., 1995, 1996). Thus under field conditions, visual stimuli would be expected to influence phase shift indirectly, and in the case of solitary insects, serve to inhibit rather than promote gregarization by providing a repulsive stimulus. In addition it should be noted that the stimuli that initiate a phase change might not be the same as those that sustain the process thereafter (Roessingh et al., 1993). Consequently, testing stimuli in isolation and out of their context might produce qualitatively different results from that evoked by a combination of stimuli.

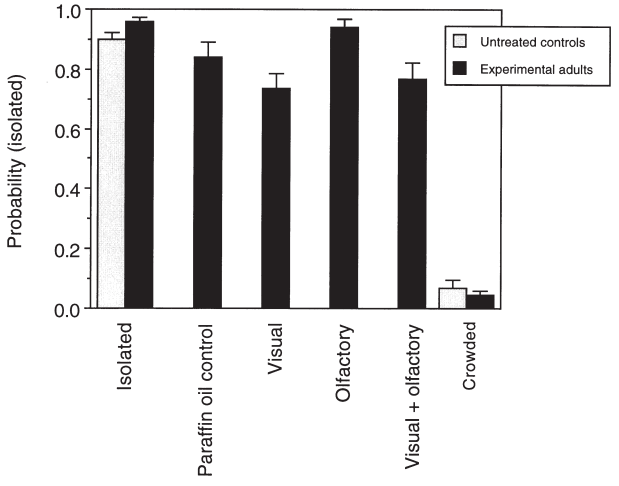

Fig. 5. Mean values (± S.E.) of *p*(isolated) for 160 experimental adults treated with visual stimuli and/or synthetic adult male aggregation pheromone blend (40 adults per treatment), and 160 control insects: 80 reared isolated and 80 reared crowded.

Although considerable work has been done on the effect on phase of olfactory stimuli provided by other locusts, many of the data are contradictory and the role of airborne volatiles in phase change is doubtful (Whitman, 1990). There is, however, relatively good evidence that aggregation, (or at least group cohesion) is promoted by volatiles from locusts and their faeces (Norris, 1963, 1970; Fuzeau-Braesch et al., 1988; Obeng-Ofori et al., 1994a, b; Torto et al., 1994; Heifetz et al., 1996; Torto et al., 1996) and this will indirectly influence locust phase state once they are brought into sufficiently close contact. The direct role of olfactory stimuli in changing phase is unclear. Electrophysiological recordings show that all active pheromone components can be detected by sensilla located on the antenna (Torto et al., 1994; Anton and Hansson, 1996; Ochieng et al., 1998), but evidence for direct effects of olfactory stimuli alone on phase is still lacking. Recently Heifetz et al. (1996) investigated a subset of the components of the adult male aggregation pheromone and concluded that they were not directly involved in changing phase. In the present paper we confirm this result for a blend of all four active components (Torto et al., 1994) of the adult aggregation pheromone.

In our experiments with synthetic adult male aggregation pheromone blend tested on nymphs and adults, no direct effect could be demonstrated, however, natural locust odours did cause behavioural gregarization of nymphs when combined with visual stimuli. Under natural conditions the two would of course exist together. Given the clear effects on the behavioural phase state of visual stimuli combined with natural locust odours, this result suggests that the synthetic pheromone blend is still incomplete, or not correctly dosed. In addition, it cannot be excluded that the two additional compounds that have no effect on aggregation do play a role as gregarizing stimuli. Alternatively, our use of adult rather than nymphal pheromone could explain our result. Even so, the synthetic adult male aggregation pheromone blend not

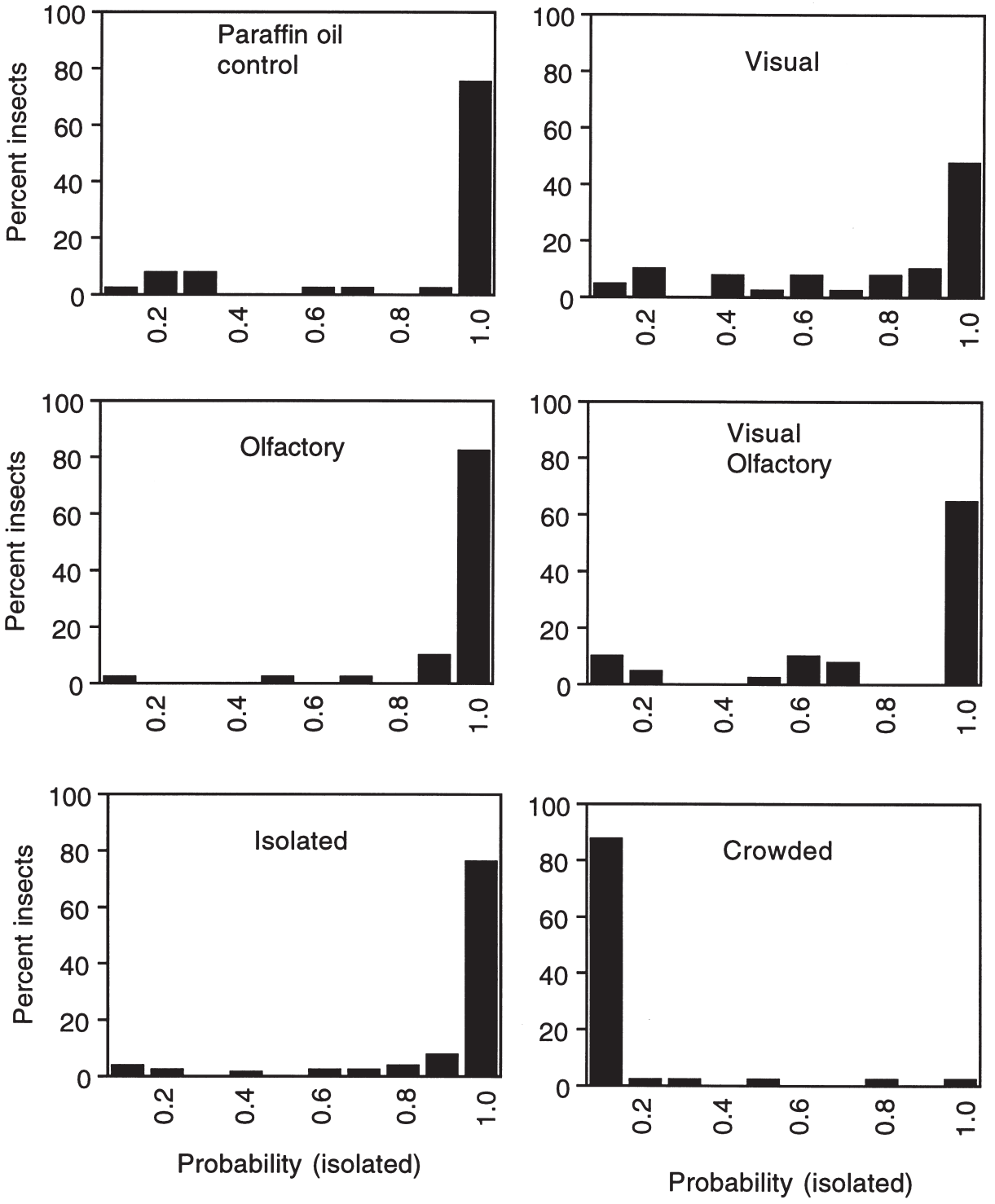

Fig. 6. Frequency histograms of the values of *p*(isolated) for 160 experimental adults treated with visual stimuli and/or a synthetic adult male aggregation pheromone blend (40 insects per treatment), and 160 additional control insects: 80 reared isolated and 80 reared crowded.

only proved ineffective on nymphs, neither did it gregarize adults. After the completion of this study a larval aggregation pheromone mixture has been published (Torto et al., 1996) and possible direct gregarizing effects of this mixture need to be evaluated.

In the present study we did not investigate chemotactile stimuli that might be present on locust cuticle. Norris (1963, 1970) carefully investigated the effects of both olfactory and chemotactile stimuli. She used the distribution of egg batches produced by gravid females as an indicator of aggregation, and concluded that chemical factors mediated cohesion rather than attraction, and that

Table 4
Analysis of variance for normal scores from rank-transformed values of *p*(isolated) of adult locusts calculated with the model in Fig. 1b

Source of variation	df	*F*	*P*
Main effects:			
Olfactory stimuli	1	0.25	0.621
Visual stimuli	**1**	**27.71**	**< 0.001**
Age	1	3.09	0.081
Sex	1	0.02	0.895
Interactions:			
Olfactory × Visual	1	0.08	0.783
Olfactory × Age	1	1.21	0.274
Olfactory × Sex	1	3.30	0.071
Visual × Age	1	0.72	0.397
Visual × Sex	1	0.01	0.903
Age × Sex	1	3.05	0.083
Olfactory × Visual × Age	1	0.19	0.660
Olfactory × Visual × Sex	1	0.54	0.465
Olfactory × Age × Sex	1	0.14	0.710
Visual × Age × Sex	1	1.47	0.228
Olfactory × Visual × Age × Sex	1	0.39	0.533
Residual	144		

Pre-reproductive and reproductive isolated-reared male and female adults were exposed to visual stimuli (10 locusts) and/or olfactory stimuli (synthetic adult male aggregation pheromone blend) and their behaviour was quantified in the bioassay arena after 4 h treatment.

effective stimuli were largely chemotactile in nature. Heifetz et al. (1996) used a Y-tube olfactometer as well as behavioural observation and in this way separated attraction from arrestment, and olfactory from chemotactile stimuli. These authors concluded that locust odour caused attraction towards the stimulus source but had no direct gregarizing effect, while chemotactile stimuli were not attractive but elicited significant behavioural gregarization. In addition it was shown by analysis of the second messenger IP_3 that cuticular hydrocarbons can indeed be detected by the antenna receptor neurons (Heifetz et al., 1997). Given these observations, stimulation by paper balls (the present experiments) or fine wires (Ellis, 1959) does not fully exclude self-stimulation by cuticular components. On a more speculative note, perhaps intensified self-grooming behaviour, which is a feature of gregarizing insects (Roessingh et al., 1993) might contribute to phase change by distribution of cuticular compounds. Further investigation of chemotactile stimuli such as cuticular hydrocarbons seems promising and is clearly needed.

It has been suggested by Haskell (1962) and Mordue-Lunz (1977) that density-dependent phase changes might be brought about by neurochemicals released in response to the intensified sensory flow under crowded conditions. The synergistic interactions of stimuli reported in this paper might be expected under this hypothesis. However, the observation that stimuli that are not locust-related (light flashes, food odours) had no significant effects on behavioural phase state indicates that the mechanism is sensory specific. Apparently, a certain amount of sensory processing, probably in the higher integrative areas of the brain, is involved before the physiological changes associated with the behavioural phase change are evoked.

Phase change has always been recognised as a complex phenomenon affecting many aspects of locust biology (Pener, 1991). Recent work on the causative factors begins to reveal that this is already true at the level of the inputs involved. Stimuli such as prolonged visual and tactile stimulation, natural locust odour, chemicals from egg pod foam (McCaffery et al., 1998) and chemotactile cues derived from the cuticle (Heifetz et al., 1996) are effective to varying degrees and at varying times in development to induce phase change. In addition, indirect effects of aggregation caused by semiochemicals (Saini et al., 1995; Rai et al., 1997) or environmental factors (Bouaïchi et al., 1996) play an important modulating role, and can have a large influence on the final outcome of the gregarization process, as indicated by simulation modelling (Collett et al., in prep.). This once more underpins the need for a multi-disciplinary approach to the problem of locust phase change.

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