Long-term memory in territorial grasshoppers

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Summary. Males of *Ligurotettix* spp. grasshoppers (Orthoptera, Acrididae, Gomphocerinae), territorial species that defend individual host plant shrubs, home to their territories by memorizing landmarks. Thus, the insects can navigate efficiently toward high quality shrubs which are chemically distinctive and recognizable via gustation, but indistinguishable from a distance.
Key words. Grasshoppers; herbivory; homing behavior; host plant chemistry; landmark navigation; *Ligurotettix*; memory; territoriality.

It is becoming increasingly apparent that learning plays a role in the feeding behavior of phytophagous insects. For example, several recent studies indicate that various species learn to associate olfactory or visual cues with food [1-4]. We now provide the first report that the vastly more sophisticated feat of learning routes between landmarks and the concomitant ability to home also occurs in some species. Our studies of *Ligurotettix* spp. grasshoppers (Orthoptera, Acrididae, Gomphocerinae) show that these insects commit an elaborate knowledge of their local landscape to long-term memory, a process enabling them to orient toward preferred host plants in an efficient manner. Such learning mechanisms would be particularly advantageous for insect herbivores that are confronted with variation in the quality of their food resources.

We obtained evidence that *Ligurotettix* grasshoppers navigate via learned routes between landmarks by observing homing orientation in two territorial species, *L. planum* and *L. coquilletti*. Many of the males in both species exhibit a high degree of site fidelity and spend most of their adult lives in one individual host plant [5-8]. Moreover, the insects often defend these sites from intrusion by conspecific males, passively via stridulation and actively via aggression [8,9]. Occasionally, and especially during agonistic encounters in *L. planum*, these sedentary individuals may leave their home sites and fly 1–10 m away to locations beyond adjoining territories. Such departures are normally followed by prompt returns, however (figs 1 and 2).

Homing events involve returns to host plants of unusually high food quality. Both *L. planum* and *L. coquilletti* are oligophagous species that feed predominantly on leaves of the Nearctic desert shrubs *Flourensia cernua* (Asterac.) and *Larrea tridentata* (Zygophyllac.), respectively [5-8,10]. Certain individual plants within stands of these shrubs harbor male and female grasshoppers each year, while

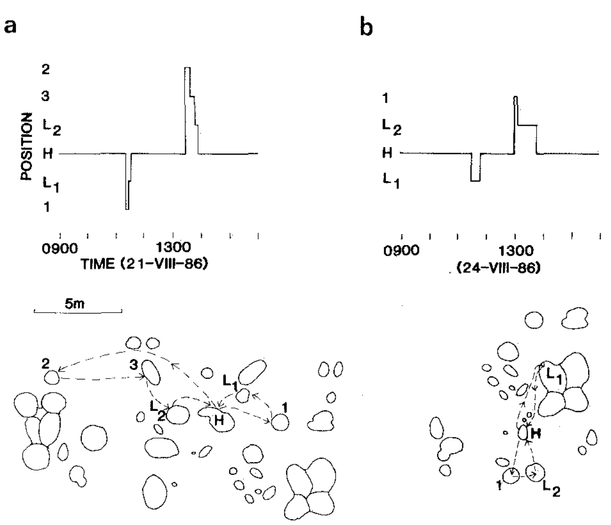

Figure 1. Four homing movements of two *L. planum* males in a field plot near Rodeo, New Mexico. Elliptical outlines represent *F. cernua* shrubs; intervening area is primarily bare ground. H is the home shrub and L_1 and L_2 are the shrubs visited immediately prior to returning to H during successive homing movements. The graphs above each map provide positions of the insects throughout the monitoring periods.

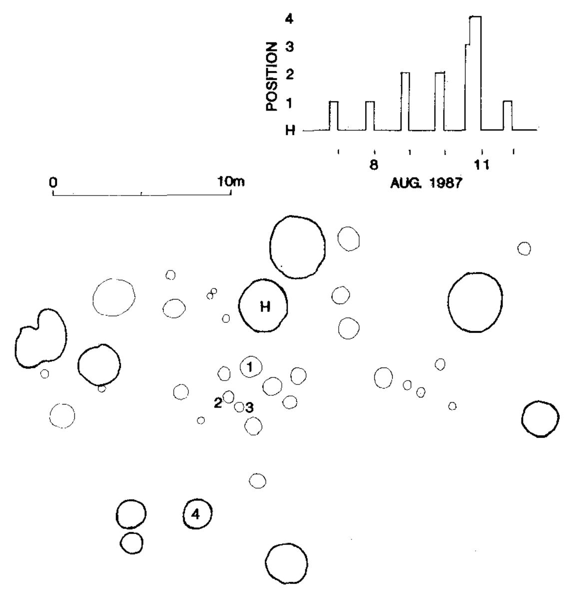

Figure 2. Homing movements over a 6-day period of an *L. coquilletti* male in a field plot at Deep Canyon, California. Thickened outlines represent *L. tridentata* shrubs; faint outlines are non-host plants. H is the home shrub. The graph above the map provides the position of the insect throughout the period.

others remain consistently vacant[7,8,11]. The occupied shrubs are neither physiognomically distinguishable nor differentiated by high leaf water or N_2 contents, yet feeding trials reveal that their foliage is preferred and that its consumption results in faster and more efficient growth[7,11-13]. Preference for particular *L. tridentata* shrubs by *L. coquilletti* can be partially explained by lower titers of a lignan catechol, nordihydroguaiaretic acid (NDGA), in the extrafoliar resin[11,12].

The grasshoppers, which are univoltine and overwinter as eggs, actively seek the high quality (preferred) shrubs early each year. In this search the insects must actually arrive at a shrub and feed or palpate to assess its quality[11]. Males that locate and occupy high quality shrubs encounter more females and mate more frequently than males situated elsewhere[14].

Methods and results

Homing in L. planum. We marked and observed the entire, natural population of *L. planum* within a 200-m^2 plot containing 51 *F. cernua*. Observations were made continuously throughout the midday (09.00–16.00 h) activity periods of the insects for 12 consecutive days during the peak in adult population. We conservatively defined homing as departing and returning, within 1 h, to a shrub otherwise occupied for > 70% of the 7-h monitoring period of a day. By these criteria, 12 of the 28 males present homed on one or more occasions. These 12 males included both dominant and subordinate individuals. Returns to home shrubs from immediately preceding locations averaged 1.5 m (range = 1–8 m), 4 × the mean nearest-neighbor distance (edge-to-edge) between host plants. Twenty-six of the 41 homing events comprised single outbound and single return flights (fig. 1 b). Return movements by many of the above insects were directed specifically to the home shrubs and were not attributable to chance. For each homing event, we considered the set (S) of potential sites (*F. cernua* shrubs only) to which the insect could have easily moved from its last location (L) prior to arriving at the home shrub (H). We restricted S to include H plus other, unobstructed shrubs within 2 m of L, since this was the mean distance of all *L. planum* flights. Thus, for the four homing events of the two males reported in figure 1, S comprised four, five, six, and three potential sites, and the respective probabilities (pr) of the males moving to H by chance were 0.25, 0.20, 0.17, and 0.33. For each male that homed, we considered the interval of one or more consecutive days during which > 70% of each monitoring period was spent in a single shrub (H) and, assuming independence of successive homing events during this interval, calculated the male's overall probability (Pr) of making chance returns to H as $Pr = \prod_{i=1}^{n} (pr)_i$, i being the i^{th} of the n successive homing events. Eight of the 12 insects observed homing had Prs < 0.05. We assumed independence of homing events because learning during the interval spent at H, the factor most likely to generate dependence, had to be ignored: the null hypothesis of chance returns obviously excluded the possibility that the insects increased their probability of returning with experience.

We also determined that homing returns did not represent mere orientation toward the nearest or largest silhouettes. For each different L visited by every homing insect prior to returning to H in the 41 homing events, we measured the distances between L and each shrub in set S and the azimuth and altitude angles that each shrub in S projected at L. The rank of H among all shrubs in S was computed for all three parameters, and we noted whether H was above or below the median rank in each case. When tabulated for all Ls of every insect, H did not rank significantly below the median for any parameter ($p > 0.05$, binomial test).

The males that homed occupied eight different *F. cernua* shrubs. These eight were all included in the set of 18 shrubs, within the plot, that harbored one or more territorial *L. planum* males during either of the two previous years. One of the homing males began occupying a different shrub in the middle of our study. This individual homed to both locations during its respective occupancies (Pr < 0.05 at both shrubs). Seven of the eight insects with Prs < 0.05 returned to H from two or more Ls (fig. 1).

Displacement experiment. We displaced 12 sedentary *L. planum* males (individuals who had not left their shrubs for > 24 h) occupying 12 different shrubs by quickly removing them in a rotated, opaque container to an artificial release perch 2–5 m from the home shrub. The perches were positioned such that they were roughly equidistant from 4–10 similarly sized host plant shrubs including the home. Two observers continuously monitored the displaced insect for 1 h from positions > 10 m distant to preclude disturbance. Eight of the insects homed within the allotted 1 h in each of two trials conducted 24 h apart in which the perch was alternately positioned on opposite sides of the home. Prs, as defined above, for these males were all < 0.05. Two additional insects only homed once. We conclude that return movements in *L. planum* are not dependent on information acquired during the outbound phase of a homing event.

Wind direction was recorded to the nearest of the 16 primary compass directions at the time of each trial and compared with the direction from the home shrub to the release perch. The angle between these two vectors averaged 79° (range = 0°–180°) in the trials where homing occurred and a statistically equivalent 56° (range = 22°–90°) in those where it did not ($p > 0.05$, Mann-Whitney U-test). That returns were not dependent on the home shrub being upwind (angle ~ 0°) from the release perch indicated strongly that olfactory cues were not used. Rather, the above findings and the observation that 1/2 of the homing returns involved a single, direct flight within 5 min of the beginning of the trial suggest that *L. planum* probably rely on knowledge of the visual landscape stored in long-term memory in order to home.

Six of the eight insects that homed twice in the above trials were then tested on a following day in a third displacement trial in which we systematically altered the profiles, via placement of *F. cernua* branches, of the home shrub and its neighbors. Three insects moved among the altered shrubs without homing, yet quickly returned once the alterations were removed 1 h later. However, the three other insects still homed during the presence of the alterations. While it remains most likely that *L. planum* males use certain visual cues to home, the specific features memorized remain unclear and may vary between individuals.

Homing in L. coquilletti. We monitored the entire *L. coquilletti* population within a 1200-m^2 plot containing 23 *L. tridentata* for two months. Due to the reduced incidence of movement in *L. coquilletti*[8] but the longer distances of individual flights, we defined homing as departing and returning, within 12 h, to a *L. tridentata* shrub otherwise occupied by the insect for > 12 h on each of 6 or more consecutive days and used 8 m in designating S (fig. 2). Our observations showed that homing movements in many *L. coquilletti* did not involve chance returns to H. Eight of the 35 males in the plot had Prs < 0.05 (Pr defined as for *L. planum*). Homing returns in these eight males were not directed preferentially toward shrubs distinguished by being relatively large or close to L ($p > 0.05$, binomial tests as used for *L. planum*). We propose that similar mechanisms may govern homing in both species.

The eight males with Prs < 0.05 were all subordinate ones who repeatedly departed their home shrubs for other *L. tridentata* or non-host plants 2–10 m distant, stridulated (to attract females) at the new site for several h, and then returned, resuming subordinate (silent) status[9]. Four different *L. tridentata* served as home shrubs. NDGA titers of three were lower than five of their six nearest neighbors, and all four were heavily occupied in two preceding years. These findings, as well as the histories of the shrubs to which *L. planum* homed, suggest that homing in both species is associated with high quality sites.

Discussion

Landmark navigation probably evolved in *Ligurotettix* due to high variation in host plant quality and the resulting skewed distribution of females. Insects departing a high quality shrub who could still return quickly would possess a selective advantage over those having to locate, by trial and error, another site with comparable resource quality and/or female numbers. Thus, our results indicate, contrary to previous opinion[5], that territorial insects may distinguish among similar sites, particularly when compelling ecological circumstances exist. Moreover, these circumstances may lead to the evolution of complex navigational abilities rivaling those previously recognized among insects only in the Hymenoptera[15–21].

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1 Prokopy, R. J., Averill, A. L., Cooley, S. S., and Roitberg, C. A., Science *218* (1982) 76.
2 Traynier, R. M. M., Physiol. Ent. *9* (1984) 465.
3 Bernays, E. A., and Wrubel, R. P., Physiol. Ent. *10* (1985) 359.
4 Papaj, D. R., Anim. Behav. *34* (1986) 1281.
5 Otte, D., and Joern, A., J. Anim. Ecol. *44* (1975) 29.
6 Greenfield, M. D., and Shelly, T. E., Anim. Behav. *33* (1985) 1192.
7 Greenfield, M. D., and Shelly, T. E., in: A Biology of Grasshoppers. Eds R. F. Chapman and A. Joern. Wiley, New York (1989) in press.
8 Shelly, T. E., and Greenfield, M. D., Behaviour (1989) in press.
9 Shelly, T. E., and Greenfield, M. D., Anim. Behav. *33* (1985) 1211.
10 Otte, D., The North American Grasshoppers, vol. 1. Harvard Univ. Press, Cambridge 1981.
11 Greenfield, M. D., Shelly, T. E., and Downum, K. R., Ecology *68* (1987) 828.
12 Chapman, R. F., Bernays, E. A., and Wyatt, T., J. chem. Ecol. *14* (1988) 561.
13 Greenfield, M. D., Shelly, T. E., and Gonzalez-Coloma, A., J. Anim. Ecol. *58* (1989) 973.
14 Shelly, T. E., Greenfield, M. D., and Downum, K. R., Anim. Behav. *35* (1987) 1200.
15 Rau, P., J. comp. Psychol. *9* (1929) 35.
16 Janzen, D. H., Science *171* (1971) 203.
17 Heinrich, B., Ecol. Monogr. *46* (1976) 105.
18 Hölldobler, B., Science *210* (1980) 86.
19 Wehner, R., in: Neuroethology and Behavioural Physiology, p. 366. Eds F. Huber and H. Markl. Springer-Verlag, Berlin 1983.
20 Gould, J. L., Science *232* (1986) 861.
21 Rosenheim, J. A., Behav. Ecol. Sociobiol. *21* (1987) 401.

0014-4754/89/080775-03$1.50 + 0.20/0