Control of Pheromone Quantity in Odor Trails of the Ant *Acanthomyops interjectus* MAYR

It is necessary for the economy of a foraging colony to adjust the number of ants leaving the nest to circumstances. After the discovery of a new food source, the returning foragers must have the ability to control the flow rate of recruits according to the quality of the bait. Since the number of workers responding to a trail increases with increasing amount of pheromone[1], this regulation can be achieved by mass-communication, that is, by the adjustment of the percentage of returning foragers that actually lay a trail after feeding at the bait[1,2]. This mechanism was considered to be sufficient to explain the findings that a *Solenopsis saevissima* colony responds faster and more strongly to high quality food sources than to poor ones[1]. However, HANGARTNER[3] demonstrated for *Solenopsis geminata* that the individual ants which do lay trails contribute to the effectiveness of the mass-communication system. They increase the continuity of their trail laid by the extruded sting with increasing starvation time of the colony, increasing quality of the food source and decreasing distance between bait and nest. Because the amount of trail substance emitted by an individual worker of this species is too minute to be measured, no direct statement could be made as to whether or not a trail-layer is also able to adjust the quantity of trail substance leaving its body per unit of time.

Acanthomyops interjectus is an ideal species in which to investigate all of these potential control mechanisms in one experiment. The trail pheromone of these ants is stored in the hind gut and emitted through the anus. The trails consist of odorous marks clearly separated from each other. If the workers are allowed to walk over a soot-coated surface, the place where a mark was set is indicated by a series of short, parallel lines created by the hairs surrounding the anal opening[4]. The soot technique therefore opens the possibility of determining whether or not an ant laid a trail on its way back to the nest, and how many marks each trail-layer set per given distance of trail. The following findings make it even possible to distinguish quantitatively between strong and weak marks. If a strong mark is made on a smoked surface, the amount of hind gut solution released is sufficient to wet the immediate environment of the spot where it was deposited and to create a circular, triangular or elliptical pattern of variable size (Figure 1, a). A weak mark, on the other hand, does not contain enough pheromone fluid to spread out on the soot and to cause such a pattern (Figure 1, b).

Five thousand to 10,000 ants were housed in a plexiglas container ($40 \times 30 \times 15$ cm) half filled with moist soil. Because they are completely subterranean in their habits[5], they were trained for 3 months to find the food (sugar water, honey, *Drosophila*) outside the nest at the end of a glass tube (20×1.5 cm) which was inserted into an excavation of the tunnel system. After this time, they were starved for 10 days. A soot-coated glass slide ($35 \times 12 \times 1$ mm) was then used as a bridge to connect the end of the glass tube with the food source (HANGARTNER[3]). The food consisted of 30 mm³ of either 0.01 or 1 *M* sucrose solution. Individual workers were allowed to find the bait and to lay a trail back to the nest after feeding there. Shortly before the returning test animals reached the artificial nest entrance, they were removed and separated from the colony for the rest of the experiment. A new slide was used for each forager. After each run, the soot coating was immediately checked under a dissecting microscope to decide whether the returning forager had laid a trail. In a positive case, the total number of marks deposited on the bridge were counted and the proportion of weak and strong marks determined.

One hundred fifty workers were tested in this way, while the concentration of the sugar water used as a bait was alternated after each single run. Subsequently, the colony was fed honey and cockroaches for 4 days. Following a second starvation period of 1 week, the whole procedure was repeated. The results of the first experiment are represented in Figure 2, a–c.

Figure 2, a shows that mass-communication also works in *Acanthomyops interjectus*. More returning foragers lay a trail after contacting the good food source than after feeding at the poor bait. The contribution of an individual worker in controlling the amount of pheromone in the trail according to the quality of the food by varying the frequency of marks can be seen in Figure 2, b. If a returning ant lays a trail, the number of touchdowns grows larger with increasing concentration of the sucrose solution. The workers that filled their crop with 1 *M* sucrose set almost twice as many odorous marks than their nestmates which were offered only 0.01 *M* sucrose solution as food. Since the variation of the frequency of marks is merely another way to control the continuity of the trail, this result is fully in accordance with the corresponding data obtained for *Solenopsis geminata*[3]. It is certainly interesting to see that 2 species, which use completely different trail-laying techniques, apply similar control mechanisms in order to achieve the same goal.

The most interesting outcome of this experiment is the one displayed in Figure 2, c. It shows that the proportion of strong marks increases with increasing food quality. This indicates that besides varying the frequency of marks, the workers are also able to adjust the amount of pheromone fluid released per mark to circumstances. HANGARTNER[3] speculated that trail-laying workers of *Solenopsis geminata* can control the quantity of pheromone leaving their body per unit of time according to the

[1] E. O. WILSON, Anim. Beh. *10*, 134 (1962).
[2] E. O. WILSON, Anim. Beh. *10*, 148 (1962).
[3] W. HANGARTNER, Z. vergl. Physiol. *62*, 111 (1969).
[4] W. HANGARTNER, J. Insect Physiol. *15*, 1 (1969).
[5] M. W. WING, Mem. Cornell Univ. agric. Exp. Stn. *405*, 1 (1968).

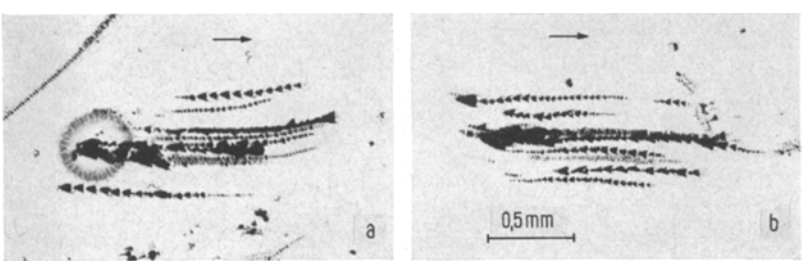

Fig. 1. Negatives of marks set by a trail-laying worker of *Acanthomyops interjectus* on a soot-coated glass plate. (a) Strong mark: the amount of hind gut solution is large enough to spread out on the soot and to create a generally circular pattern. (b) Weak mark: the quantity of pheromone fluid emitted is not sufficient to spread out. Only the lines drawn by the tip of the abdomen and by the hairs are seen. The arrows indicate the direction of running.

quality of the food source. For *Acanthomyops interjectus*, this prediction now proves to be right, at least for 2 baits of different sweetness.

Since the results of the repetition were almost identical with the ones just described, we can conclude that trail-laying in *Acanthomyops interjectus* is not an all-or-none response. Each trail-layer can regulate its contribution to the trail in 2 ways, i.e., by varying the frequency of odorous marks and by controlling the amount of pheromone fluid released per mark. The adjustment of the pheromone quantity in the trail to the quality of the food in order to regulate the number of workers that leave the nest is therefore threefold and should prove to be a highly effective mechanism to establish optimal foraging economy[6].

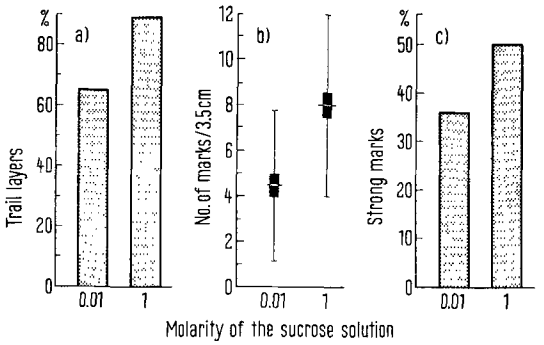

Fig. 2, a–c. The mechanisms used by *Acanthomyops interjectus* to control the amount of pheromone in trails connecting the nest with 2 food sources of different quality (0.01 and 1 *M* sucrose solution). (a) Adjustment by varying the percentage of trail-layers. Number of ants tested: 1 *M* sucrose = 75; 0.01 *M* sucrose = 75. (b) Adjustment by varying the number of marks set by an individual forager per 3.5 cm of its trail. Number of trails evaluated: 1 *M* sucrose = 67; 0.01 *M* sucrose = 49. Indicated are mean, standard deviation and standard error. (c) Adjustment by varying the percentage of strong marks. Total number of marks: 1 *M* sucrose = 536; 0.01 *M* sucrose = 217. The differences in all 3 cases are significant at the 0.1% level (χ^2-test and *t*-test, respectively).

Zusammenfassung. Die Menge Spurpheromon in den Duftspuren der Ameisenart *Acanthomyops interjectus* wird der Qualität des Futters angepasst. Mit zunehmender Konzentration des gebotenen Zuckerwassers erhöht sich der Prozentsatz spurenlegender Ameisen. Jedes spurenlegende Tier setzt zudem mehr Duftmarken pro Wegeinheit. Gleichzeitig nimmt auch die Menge Spursubstanzlösung zu, die pro Marke ausgeschieden wird. Da die Anzahl der von einer Duftspur angelockten Nestinsassen mit zunehmender Pheromonmenge ansteigt, wird angenommen, dass dieser dreifache Kontrollmechanismus die Zahl der ausschwärmenden Arbeiterinnen der Futterqualität entsprechend reguliert.

W. Hangartner[7]

The Biological Laboratories, Harvard University, Cambridge (Massachusetts, USA), 21 December 1969.

[6] This work was supported by U.S. National Science Foundation Grant No. GB7734 (Dr. E. O. Wilson sponsor), and a grant from the Swiss National Science Foundation. I thank Dr. E. O. Wilson and Miss N. K. Lind for reading the manuscript.

[7] Present address: Biologisches Laboratorium, Dr. R. Maag AG, CH-8157 Dielsdorf (Switzerland).

Infection with Viruses of the Tacaribe Group in Thymectomized Mice

Lesions dependent on the presence of antibodies or sensitized cells may be decreased or prevented in mammals by neonatal thymectomy. This was demonstrated for lymphocytic choriomeningitis[1], and Argentine hemorrhagic fever (Junin virus)[2]. In this preliminary work we report that the characteristic survival of thymectomized mice infected with Junin virus is equally characteristic of thymectomized mice infected with other members of the Tacaribe group of virus[3,4], with one exception.

Rockland mice, a strain in which wasting disease following thymectomy is delayed[5], were thymectomized[6] within 24 h of birth and inoculated intracerebrally within 6 h with 0.02 ml of mouse brain homogenate containing 1000 LD/50 doses of one of the following members of the Tacaribe group of virus[3,4]: Junin (RC strain), Machupo (Carvallo strain), Tacaribe (TRVL 11573 strain), Pichinde (provided by Dr. C. San Martin[4], who isolated this virus in Colombia from *Oryzomis abbigularis*), and Amapari (L17.15B strain). Nonthymectomized new-born Rockland mice of the same age were injected intracerebrally with similar amount of virus. All mice were examined daily for neurological symptoms characteristic for these viral infections[2]. Thymectomized mice which died spontaneously or when the period of observation was complete were examined macroscopically and microscopically to determine whether thymectomy was complete. Mice with remains of thymus were rejected from the results. Almost all thymectomized mice infected with Junin, Machupo, Tacaribe, or Pichinde virus survived (98%, 100%, 97% and 96%, respectively); all control mice died between the 5th and 21st day after infection (Table) and had neurological symptoms typical of virus infection[2]. In contrast, neither thymectomized nor nonthymectomized mice infected with Amapari virus survived longer than 20 days.

We reported that Junin virus could be recovered from the brains of surviving thymectomized mice 50 days after infection. Serum antibodies were never detected in these mice; spleen from immune, but not normal mice inhibited the effect of thymectomy but immune serum did not[5]. These findings demonstrate the importance of delayed hypersensitivity in the pathogenesis of infections with Junin virus, and strongly suggest the same mechanism in infections with viruses of the same group. In this study

[1] R. H. Levey, N. Trainin, L. W. Law, P. H. Black and W. P. Rowe, Science *142*, 483 (1963).
[2] G. A. Schmunis, M. C. Weissenbacher and A. S. Parodi, Arch. ges. Virusforsch. *21*, 201 (1967).
[3] R. M. Taylor, *Catalogue of Arthropod-Borne Viruses of the World* (Public Health Service publication 1760, USA 1968).
[4] H. Trapido and C. San Martin, in preparation.
[5] M. C. Weissenbacher, G. A. Schmunis and A. S. Parodi, Arch. ges. Virusforsch. *26*, 63 (1969).
[6] W. Dischler and C. Rudali, Revue fr. Étud. clin. biol. *6*, 88 (1961).