# Oviposition Process of *Solenopsis invicta* (Hymenoptera: Formicidae) Queens

R. K. VANDER MEER[1] AND L. MOREL[2]

Center for Medical, Agricultural, and Veterinary Entomology, USDA–ARS, 1600 SW 23rd Drive, Gainesville, FL 32608

**ABSTRACT** The oviposition process of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is described as a succession of four events forming one egg-laying cycle. Each time an egg is laid, the vulva opens and the sting is fully extended. When the vulva closes, the egg is forced to the base of the sting, whereupon the sting is usually retracted across the egg. This phenomenon has been consistently observed for queens from monogynous and polygynous *S. invicta* colonies. Zero to four eggs can be laid for each opening of the vulva. Multiple egg deposition is more frequent for monogyne queens, and it generally follows an oviposition cycle in which no egg is laid. The egg-laying cycles were evenly distributed during the observation periods, regardless of the total number of eggs laid, thereby suggesting that oviposition is a continuous process in *S. invicta*.

**KEY WORDS** *Solenopsis invicta*, oviposition, sting apparatus, monogyny, polygyny

Host selection and food provisioning associated with oviposition have been investigated in a number of species of solitary and social Hymenoptera (for review, see Hinton 1981). Social regulation of egg laying or of the oviposition rate in both queens and workers has been studied in a number of ant species (for a review see Tschinkel 1988). However, little attention has been given to the behavior of the queen during the egg-laying process. Therefore, we made detailed observations of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) queens while ovipositing. *S. invicta* is a South American ant that has attained major pest status in the southern United States (Lofgren et al. 1975). Both monogyne (a single queen per nest) and polygyne (multiple queens per nest) populations of this prolific species are known in the United States. Each monogyne colony can contain up to 250,000 workers; however, worker numbers are even greater in polygyne populations where nest densities are greater and where each nest may contain several hundred queens (Glancey et al. 1975). In addition to possessing a different social structure, polygyne and monogyne colonies differ by the size distribution of workers, nest density (Greenberg et al. 1985), and colony proliferation and perpetuation (Glancey and Lofgren 1988; Vargo and Porter 1989). The fecundity of *S. invicta* queens is high compared with other ant species. A mature (i.e., ≥3-yr-old) monogyne queen can pro-

duce her own weight in eggs every 24 h (Tschinkel 1988). This probably contributes to the remarkable ecological success of this species in the southern United States.

The reproductive system of *S. invicta* (Hermann and Blum 1965) and the sting apparatus with its associated glands (Callahan et al. 1959) have been described. The evolution of the different elements of the reproductive system and sting apparatus can be traced through the hymenopteran order (Scudder 1961). The ant sting has evolved from the ancestral ovipositor. It is separated from the vulva, with the eggs no longer deposited through the sting, as is the case for the ovipositor. Associated with the sting are the poison and the Dufour's glands, both of which empty through the sting bulb (see Hermann and Blum 1981 on the evolution of the sting apparatus and associated glands in Hymenoptera). The flow of exocrine products of each gland is controlled by independent musculatures (Callahan et al. 1959, Billen 1987).

We used this anatomical and morphological background to study by direct observation the oviposition behavior of *S. invicta* queens. We report here a detailed description of the oviposition process for queens from the monogyne and polygyne social forms of *S. invicta.*

## Materials and Methods

**Source of Queens.** Observations were conducted on *S. invicta* queens from colonies collected in north central Florida or from laboratory colonies reared from newly mated queens collected near Gainesville, FL. All colonies were maintained in the laboratory for

[1] Corresponding author, e-mail: bobvm@ufl.edu.
[2] Current address: Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL 32610.

several months before use (for description of the rearing technique, see Banks et al. 1981). Queens selected for this study were from colonies with abundant production of all stages of brood.

Twenty-nine observations were made on 12 monogyne queens with one to four observations per queen. Thirty-two observations were made on multiple queens from five colonies. The number of queens in the polygyne colonies ranged from five to 109; however, only five to 10 queens per colony were tested. Each polygyne queen was tested only once. One monogyne queen and two polygyne queens did not lay any eggs during the observation period, bringing the number of useful observations to 28 and 30 for monogyne and polygyne queens, respectively.

**Observation Procedure.** For each test, the queen, five workers located near her, and five fourth instars and/or prepupae were removed from the colony. The queen was weighed with an analytical balance (Mettler H51Ar, Mettler Scientific, Highstown, NJ) and placed into a 100-$\mu$l glass insert contained in a vial (Sun Brokers, Wilmington, NC). The workers and brood were then placed with the queen in the insert to minimize the stress on the queen due to isolation. Observations of the queen were made under a dissection microscope for the next 2 h. The following events were timed and recorded: 1) extrusion of the sting for at least half its length; 2) presence of a venom droplet on the sting; 3) opening of the vulva; 4) appearance of one or more eggs; 5) withdrawal of the sting into the sting chamber; and 6) miscellaneous events, such as defecation. At the end of the 2-h observation period, the workers and brood were discarded, and the queen was returned to its colony.

**Data Analysis.** The average frequencies of egg deposition after each opening of the vulva were compared between monogyne and polygyne queens by a chi-square test. The coefficient of linear regression of the number of openings of the vulva leading to zero, one, or two eggs with the weight of the queens, and with the total number of eggs produced in the 2-h observation was calculated independently for monogyne and polygyne queens. A sequential analysis of the number of eggs laid at the same time was performed using a first order Markov model (Fagen and Young 1978). This analysis tested whether the event of laying "x" eggs at the same time has any effect on the number "y" of eggs laid immediately afterward (x and y are between 0 and 4). The data for monogyne and polygyne queens have been treated independently. The "3" and "4 eggs per vulva opening" events were pooled, because the number of transitions observed for these events were small. A chi-square goodness-of-fit test was used to compare observed transitions (x,y) with expected transitions (x,y). In addition, the occurrence of each individual transition (x,y) was compared with chance expectation by computing Y:

$$Y = [\text{observed}_{(x,y)} - \text{expected}_{(x,y)}] / (\text{expected}_{(x,y)})$$

If $/Y/ > (\chi^2_{0.05})/R^2$ ($R$ is the number of possible events), then the transition (x,y) occurs at a frequency that differs from chance expectation at $P <$ 0.05. If $Y < 0$, event x is said to inhibit event y, whereas if $Y > 0$, event x is said to direct event y.

The distribution of egg laying through time was studied using the following procedure: the time period between the 30th and the 90th minute (mid-hour) for each observation was divided into 5- and 10-min intervals for monogyne queens and polygyne queens, respectively. The longer intervals were needed for polygyne queens to ensure at least one egg deposition per interval. The average number of eggs laid in each interval (x) and its variance ($s^2$) were calculated for each observation. The null hypothesis that there was no significant difference in the number of eggs laid within each interval was tested using the following chi-square test:

$$\chi^2 = (n - 1) \times s^2/x$$

where n is the number of intervals. Only the data obtained between the 30th and 90th min were retained to attenuate the effect of disturbance (first half hour) and diminishing energy reserve of the queen (last half hour). In most instances, the egg-laying rate during the first and last half hour of each 2-h period was lower than the middle hour. If the first egg laid by the queen was after 30 min, or if <10 eggs were laid during the observation period, the data were not used. The resulting observation sample size was 27 monogyne and 23 polygyne queens.

## Results

The monogyne queens observed for oviposition weighed from 11.58 to 25.5 mg (mean ± SE, 19.61 ± 0.73 mg; n = 29). The weight of polygyne queens observed ranged from 8.21 to 16.41 mg (11.91 ± 0.33 mg; n = 30). The polygyne queens were taken from five colonies, where all queens weighed from 6.65 to 16.41 mg. The weights of monogyne queens were significantly higher than polygyne queens (two-tailed $t$ = 9.763, df = 56, $P$ < 0.0001) as expected from previous literature (Vander Meer et al. 1992). Correspondingly, the number of eggs laid per queen in a 2-h period was significantly higher (two-tailed $t$ = 8.557, df = 55, $P$ < 0.0001) for monogyne queens (149 ± 14.68; n = 27) versus polygyne queens (25.50 ± 3.63; n = 30).

**Description of Behaviors during an Oviposition Cycle.** The description of behaviors associated with oviposition was based on 2-h observations of 28 monogyne and 32 polygyne *S. invicta* queens. The egg-laying process was basically the same for the two forms; therefore, unless otherwise specified, the following results apply for both monogyne and polygyne queens. When the queen is not laying eggs, its sting is withdrawn into the sting chamber, and its vulva is closed. In some cases, the tip of the sting is visible. Egg deposition can be described by the following behavioral sequence (Fig. 1):

1. Extrusion of the sting. The sting was always extruded to its full length in one continuous rapid motion. The extension was sometimes as-
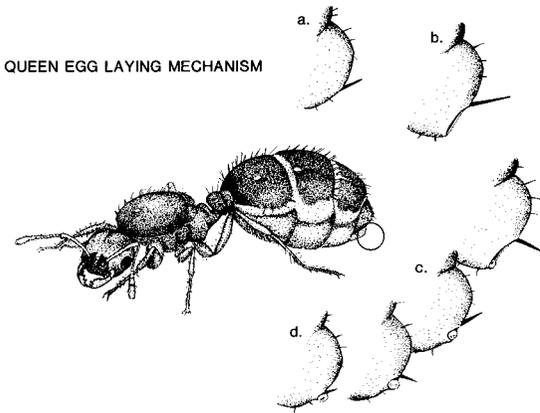
QUEEN EGG LAYING MECHANISM



**Fig. 1.** Egg-laying mechanism of *S. invicta*. (a) Partial sting extension. (b) Full extension of sting and opening of the vulva. (c) Wavelike abdominal contractions force an egg out of the oviduct and moved by the closing of the vulva onto a track formed by the sting (series of three overlapping drawings). (d) Sting is withdrawn across the egg.

sociated with the formation of a droplet of clear liquid on the distal part of the sting, probably venom (Fig. 1a).

2. Opening of the vulva. The vulva opened ventrally to the sting along with wave-like contractions of the abdomen. Sometimes, the abdominal contractions produced a succession of wide openings and closings of the vulva (Fig. 1b).
3. Egg deposition. One egg (or more; see below) on the ventral side of the vulva. The complete closing of the vulva forced the egg(s) to the base of the sting (Fig. 1c, series of three drawings).
4. Sting retraction. The sting retracted to its recessed position, generally across the egg(s) (Fig. 1d). In some cases, however, the egg was removed by a worker before the sting was retracted. Most of the time, the egg stuck to the queen's cuticle with other eggs previously laid. They were removed by workers in clusters of three to five eggs. Eggs may be removed much faster in nature, because in a normal colony situation many more workers would be available to remove oviposited eggs.

This four-event behavioral cycle could be shortened and stopped after any of the events. For example, when the egg-laying rate was high, it was common for the sting to remain extruded after an egg was laid. When this happened, behaviors 2 and 3 might be repeated up to 10 times before the sting assumed its recessed position. The duration of one egg-laying cycle (steps 1–4) was relatively short, generally <10 s., regardless of the queen egg-laying rate and whether she was from a monogyne or polygyne colony. However, the duration of time between the deposition of two successive eggs (behavior 3) was variable: The average minima and maxima of the egg-laying intervals were different between monogyne and polygyne queens (Table 1). This corresponded to differences in oviposition rates in the two forms (Vander Meer et al.

**Table 1. Minima and maxima of time intervals (seconds) between sting extrusions by monogyne and polygyne queens**

| | Monogyne queens (n = 28) | | Polygyne queens (n = 30) | |
|---|---|---|---|---|
| | Min | Max | Min | Max |
| Mean | 14 | 200 | 118 | 759 |
| SD | 8 | 163 | 135 | 566 |
| Range | 6–48 | 51–835 | 6–639 | 134–2,995 |

1992). However, comparison of the minima range shows that polygyne queens are able to lay eggs as fast as monogyne queens (6-s minimum in both cases).

One queen from a polygyne colony (16.41 mg, the largest polygyne queen tested) laid three eggs (of a total of 85 during the 2-h observation period) without extending her sting. It has not been possible to correlate this rare mode of egg laying with any monitored condition or variable.

Defecation was observed during four observations on monogyne queens. The egg-laying process was interrupted until the workers completely removed the excrement.

**Number of Eggs Laid during One Oviposition Cycle.** The number of eggs laid during one oviposition cycle varied from 0 to 4. The whole cycle was sometimes completed without a single egg being laid, or on other occasions multiple eggs (up to 4) occurred at the same time. If they occurred successively without retraction of the sting, they were considered to be eggs laid during incomplete cycles and were not included in this analysis. The average frequency of a queen depositing zero, one, two, three, or four eggs (Table 2) was significantly different for monogyne and polygyne queens ($\chi^2 = 19.64$, df = 4, $P < 0.005$). Polygyne queens laid eggs singly more often than monogyne queens (86.25 versus 61.10%, respectively; $P < 0.001$). The number of times a queen (monogyne or polygyne) laid zero, one, or two eggs at the same time was not correlated with the total number of eggs laid by that queen. The sequential analysis of data on the number of eggs laid at the same time gave a chi-square goodness-of-fit that was significant for both monogyne ($\chi^2 = 515.02$, df = 9, $P < 0.001$) and polygyne queens ($\chi^2 = 242.51$, df = 9, $P < 0.001$). Therefore, the succession of the number of eggs laid at the same time does not occur at random. This is further confirmed by the comparison of each transition to chance expectation (Fig. 2). It seems that multiple eggs (two, three, or four) occur most frequently subsequent to egg-laying cycles without egg deposition. In contrast, the

**Table 2. Frequency (%) of the number of eggs laid at the same time (0–4) by monogyne (MQ) and polygyne (PQ) queens. Data are means**

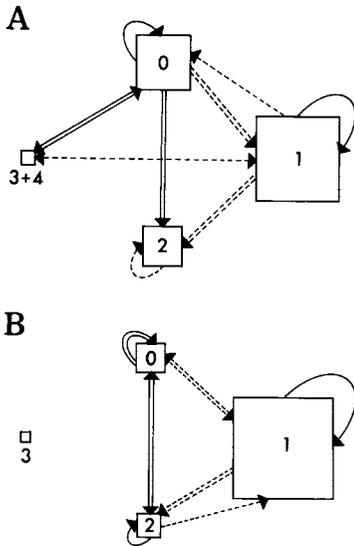| Eggs laid | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| MQ | 19.4 | 63.3 | 14.2 | 1.4 | 0.5 |
| SD | 15.0 | 19.3 | 8.8 | 2.4 | 1.4 |
| PQ | 3.9 | 90.3 | 5.2 | 0.6 | 0 |
| SD | 7.1 | 14.3 | 9.3 | 1.5 | 0 |

**A**



**B**



**Fig. 2.** Sequential analysis of the number of eggs deposited per vulva opening. The observed number of eggs (zero–four) laid during a single vulva opening is represented by the square with a surface proportional to the frequency of the act. Arrows between squares indicate when a succession between two acts occurred at a frequency that differed from chance expectation at $P < 0.05$ (single lines) or $P < 0.01$ (double line). Continuous lines indicate when successions occurred more frequently than chance expectation; dotted lines indicate when successions occurred less frequently than chance expectation. (A) Monogyne queens (28 observations; 4,514 vulva openings). (B) Polygyne queens (30 observations; 735 vulva openings).

oviposition of a single egg seems to decrease the probability of laying multiple eggs.

**Distribution of Egg-Laying Cycles over Time.** The time distribution of egg-laying cycles was analyzed for queens, which laid >10 eggs and began oviposition before 30 min. Each observation period was divided into appropriate intervals (5 or 10 min; see Materials and Methods). The number of eggs laid during each interval was analyzed by chi-square test with the null hypothesis that there was no significant difference in the number of eggs laid during each interval (see Materials and Methods). The chi-square test was significant for only one of 28 monogyne queens ($\chi^2 = 32.3$, df = 11, $P < 0.005$) and one of 23 polygyne queens ($\chi^2 = 11.12$, df = 5, $P < 0.05$). These two queens did not have any special features in terms of their weight or the total number of eggs they laid. For the 49 other cases, the egg-laying cycles were evenly distributed in time.

## Discussion

Our study provides the first detailed description of oviposition behavior in an ant species. The overall process, described as a succession of four events [1) extrusion of the sting, 2) opening of the vulva, 3) appearance of the egg(s), and 4) retraction of the sting] was the same in monogyne and polygyne *S.*

*invicta* queens. The same overall behavior was observed for pharaoh ant, *Monomorium pharaonis* (L.), queens (L.M., unpublished data), and it may apply to all ant species with a sting.

Vander Meer and Morel (1995) suggested that the involvement of the sting in the egg-laying process of ant queens provides an excellent mechanism for the release of queen-derived exocrine products onto the eggs, as well as throughout the colony. This has potential ramifications in hymenopteran evolution, caste determination and regulation, control of reproductives, and nestmate recognition. The Dufour's and venom glands play a major role in defense and pheromonal communication in social Hymenoptera (Hermann and Blum 1981). In fire ant workers, the Dufour's gland is the source of the recruitment pheromone (Wilson 1959), which elicits a variety of behaviors, including worker attraction, orientation induction, orientation, and alarm (Wilson 1962, Vander Meer 1986). The Dufour's gland of queens probably has the same morphology and ultrastructure as workers and other ant species (Billen 1986). Queens have a well-developed poison gland and poison sac, which contains antimicrobial piperidine alkaloids (Obin and Vander Meer 1985, Brand et al. 1973), as well as a pheromone that attracts workers (Vander Meer et al. 1980). Our results also provide for the first time in a social hymenopteran species, a link between the ovipositor and sting apparatus along with their associated glands.

The distribution of egg deposition in time was uniform both in monogyne and polygyne queens. The oviposition process itself is relatively constant in duration. The intervals between egg deposition increase when the egg-laying rate decreases (Vander Meer et al. 1992), but they were constant for a given observation period. This confirms the observations of Tschinkel (1988) that oviposition is a continuous process in *S. invicta* monogyne queens. It also demonstrates that the process is the same for polygyne queens in spite of their much lower oviposition rate. Both monogyne and polygyne fire ant queens deserve the appellation of "egg-laying machines," because not only is oviposition their main activity in the colony but also the process is periodical and mechanical in nature.

A difference was found between the oviposition mechanism of monogyne and polygyne queens. Polygyne queens were more consistent in terms of the number of eggs laid at each oviposition cycle (Fig. 2). Most of the time, they laid one egg at a time. However, monogyne queens more frequently laid multiple eggs at a time, usually after an oviposition cycle without an egg laid. Although the number of occurrences of multiple eggs was not correlated with the total number of eggs laid, it is likely that the irregular laying pattern of monogyne queens was due to the greater activity of their ovaries and the greater number of mature ovocytes present at the same time. The smaller size and fecundity of polygyne queens is well established (Fletcher et al. 1980, Vargo and Fletcher 1989, Vander Meer et al. 1992). Functional differences in fire ant polygyne and monogyne queens were shown for the

production of a dealation inhibitory pheromone (Willer and Fletcher 1986) and for their egg-producing ability (Vander Meer et al. 1992). How the different social structures in polygyne and monogyne colonies translate into these functional differences constitutes a challenging problem yet to be solved in *S. invicta.*

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## References Cited

Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants, pp. 1–9. U.S. Dep. Agriculture, Sci. Educ. Admin. AAT-S-21.

Billen, J.P.J. 1986. Comparative morphology and ultrastructure of the Dufour's gland in ants Hymenoptera: Formicidae). Entomol. Gen. 11: 165–181.

Billen, J.P.J. 1987. New structural aspects of the Dufour's and venom glands in social insects. Naturwissenschaften 74: 340–341.

Brand, J. M., M. S. Blum, and M. R. Barlin. 1973. Fire ant venom: intraspecific and interspecific variation among castes and individuals. Toxicon 11: 325–331.

Callahan, P. S., M. S. Blum, and J. R. Walker. 1959. Morphology and histology of the poison gland and sting of the imported fire ant (*Solenopsis saevissima v. richteri* Forel). Ann. Entomol. Soc. Am. 52: 573–590.

Fagen, R. M., and D. Y. Young. 1978. Temporal patterns of behaviors: durations, intervals, latencies and sequences, pp. 79–114. *In* P. W. Colgan [ed.], Quantitative ethology. Wiley, New York.

Fletcher, D.J.C., M. S. Blum, T. V. Whitt, and N. Temple. 1980. Monogyny and polygyny in the fire ant, *Solenopsis invicta.* Ann. Entomol. Soc. Am. 73: 658–661.

Glancey, B. M., C. E. Stringer, C. H. Craig, and P. M. Bishop. 1975. An extraordinary case of polygyny in the red imported fire ant. Ann. Entomol. Soc. Am. 68: 922.

Glancey, B. M., and C. S. Lofgren. 1988. Adoption of newly-mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. Fla. Entomol. 71: 581–587.

Greenberg, L., D.J.C. Fletcher, and S. B. Vinson. 1985. Differences in worker size and mound distribution in monogynous and polygynous colonies of the fire ant, *Solenopsis invicta* Buren. J. Kans. Entomol. Soc. 58: 9–18.

Hermann, H. S., and M. S. Blum. 1965. Morphology and histology of the reproductive system of the imported fire ant queen, *Solenopsis saevissima richteri.* Ann. Entomol. Soc. Am. 58: 81–89.

Hermann, H. S., and M. S. Blum. 1981. Defensive mechanisms in the social hymenoptera, pp. 78–198. *In* H. S. Hermann [ed.], Social Hymenoptera. Academic, New York.

Hinton, H. E. 1981. Biology of insect eggs, vol. II. Pergamon, London, United Kingdom.

Lofgren, C. S., W. A. Banks, and B. M. Glancey. 1975. Biology and control of imported fire ants. Annu. Rev. Entomol. 29: 1–30.

Obin, M. S., and R. K. Vander Meer. 1985. Gaster flagging by fire ants (*Solenopsis* spp.): functional significance of venom dispersal behavior. J. Chem. Ecol. 11: 1757–1768.

Scudder, G.G.E. 1961. The comparative morphology of insect ovipositor. Trans. R. Entomol. Soc. Lond. 111: 405–467.

Tschinkel, W. T. 1988. Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta.* Physiol. Entomol. 13: 327–350.

Vander Meer, R. K., and L. Morel. 1995. Ant queens deposit pheromones and antimicrobial agents on eggs. Naturwissenschaften 82: 93–95.

Vander Meer, R. K., B. M. Glancey, C. S. Lofgren, A. Glover, J. Tumlinson, and J. Rocca. 1980. The poison sac of red imported fire ant queens: source of a pheromone attractant. Ann. Entomol. Soc. Am. 73: 609–612.

Vander Meer, R. K. 1986. The trail pheromone complex of *Solenopsis invicta* and *Solenopsis richteri*, pp. 201–210. *In* C. S. Lofgren and R. K. Vander Meer [eds.], Fire ants and leaf-cutting ants: biology and management. Westview Press, Boulder, CO.

Vander Meer, R. K., L. Morel, and C. S. Lofgren. 1992. A comparison of queen oviposition rates from monogyne and polygyne fire ant, *Solenopsis invicta*, colonies. Physiol. Entomol. 17: 384–390.

Vargo, E. L., and D.J.C. Fletcher. 1989. On the relationship between queen number and fecundity in polygyne colonies of the fire ant *Solenopsis invicta.* Physiol Entomol. 14: 223–232.

Vargo, E. L., and S. D. Porter. 1989. Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 82: 307–313.

Willer, D. E., and D.J.C. Fletcher. 1986. Differences in inhibitory capacity among queens of the ant *Solenopsis invicta.* Physiol. Entomol. 11: 475–482.

Wilson, E. O. 1959. Source and possible nature of the odour trail of the fire ants. Science (Wash., D.C.) 192: 643–654.

Wilson, E. O. 1962. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass foraging. 2. An informational analysis of the odour trail. 3. The experimental induction of social responses. Anim. Behav. 10: 134–164.