

Report

A Mechanical Signal Biases Caste Development in a Social Wasp

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Summary

Understanding the proximate mechanisms of caste development in eusocial taxa can reveal how social species evolved from solitary ancestors [1]. In *Polistes* wasps, the current paradigm holds that differential amounts of nutrition during the larval stage cause the divergence of worker and gyne (potential queen) castes [2]. But nutrition level alone cannot explain how the first few females to be produced in a colony develop rapidly yet have small body sizes and worker phenotypes [3, 4]. Here, we provide evidence that a mechanical signal biases caste toward a worker phenotype. In *Polistes fuscatus*, the signal takes the form of antennal drumming (AD), wherein a female trills her antennae synchronously on the rims of nest cells while feeding prey-liquid to larvae [5]. The frequency of AD occurrence is high early in the colony cycle, when larvae destined to become workers are being reared, and low late in the cycle, when gynes are being reared [6]. Subjecting gyne-destined brood to simulated AD-frequency vibrations caused them to emerge as adults with reduced fat stores, a worker trait [7]. This suggests that AD influences the larval developmental trajectory by inhibiting a physiological element that is necessary to trigger diapause, a gyne trait [8].

Results

Brillet et al. [9] first suggested for *Polistes dominula* that vibrations somehow help prepare workers for their future status as dominated subordinates. Jeanne [10] recently expanded on this with a mechanical-switch hypothesis, proposing that vibrations issued in the context of larval feeding bias [11] development of the recipients toward worker-like traits. We tested the mechanical-switch hypothesis by subjecting three late-season, gyne-producing *P. fuscatus* colonies with low natural rates of performance of antennal drumming (AD) [6] to high rates of simulated AD transmitted to the nest by a piezoelectric (piezo) device (Figure 1). Three others received random-frequency pulses of similar energy intensity from an attached piezo for the same duration every day for a period encompassing the egg-to-adult development time of *P. fuscatus* [12]. All females emerging from the treated nests were frozen for analyses of their fat stores, a physiological measure of diapause potential, which is a robust gyne trait in *Polistes* [7, 8, 13] (see

Experimental Procedures). The subset of the wasps that were third instar or younger when the piezo treatment started were analyzed separately in order to distinguish the effect of treatment on younger larvae from that on older larvae and pupae. Newly emerged wasps from three untreated, late-season, field-collected colonies were also analyzed for their fat stores. Laboratory colonies were videotaped to obtain frequencies of AD behavior and of feeding prey-liquid to larvae. All newly emerged wasps were analyzed for treatment effects on body-size parameters (Figure S3, available online). In a different experiment with a separate set of colonies, we analyzed effects on newly emerged females that were pupae when first subjected to the treatment (Supplemental Information).

Mixed model analysis of variance adjusted for random nest-to-nest variation showed that treatment significantly affected percentage of fat stores ($n = 33$ wasps, 6 colonies, $F_{1,4} = 14.2$, $p = 0.019$, Figure 2; power = 0.85, see Supplemental Information). Newly emerged wasps from colonies that received simulated AD-frequency pulses had significantly lower fat stores than those from colonies that received random-frequency pulses (Figure 2). None of the other colony-wide factors—worker-to-larva ratio, liquid-feeding frequency, or frequency of AD behavior—influenced percentage of fat stores (Table 1). When vibration treatment was analyzed with each colony-wide factor and with the interaction of treatment and factor, neither the interaction nor the second factor significantly influenced percentage of fat stores. Percentage of fat stores of newly emerged wasps from colonies that received random-frequency pulses did not differ significantly from those from untreated late-season field colonies ($t_6 = 0.47$, $p = 0.46$), but those from colonies that received AD-frequency pulses did ($t_6 = 3.25$, $p = 0.018$) (Figure 2).

In a separate experiment (Supplemental Information), vibration treatment had no effect on the fat stores of newly emerged wasps that were pupae at the start of the piezo treatment ($F_{2,10} = 0.34$, $p = 0.72$) (Figure S2).

For the subset of newly emerged wasps that were in the third larval instar or below at start of the piezo treatment, the effect of treatment was even stronger ($n = 20$ wasps, 6 colonies, $F_{1,4} = 35.8$, $p = 0.004$, Figure 2). Again, neither worker-to-larva ratio, feeding frequencies, nor frequencies of wasp AD behavior significantly affected percentage of fat stores (Table 1).

Treatment and control colonies did not differ in worker-to-larva ratios ($F_{1,4} = 0.01$, $p = 0.92$) (Table 2), dry weight ($F_{1,4} = 0.33$, $p = 0.59$) (Table 2), or body size (Table S1) of newly emerged wasps or the frequencies with which their adults drummed ($F_{1,4} = 1.7$, $p = 0.26$) or fed prey-liquid to larvae ($F_{1,4} = 0.06$, $p = 0.82$).

A reanalysis of the data after excluding a potential outlier (colony 1, Table 2) showed that treatment still significantly affected percentage of fat stores for the whole set ($n = 32$ wasps, 5 colonies, $F_{1,3} = 12.8$, $p = 0.03$) and for the subset ($n = 19$ wasps, 5 colonies, $F_{1,3} = 34.7$, $p = 0.009$) of newly emerged wasps.

Discussion

We conclude that subjecting larvae in gyne-producing nests to simulated AD vibrations caused adults to emerge with

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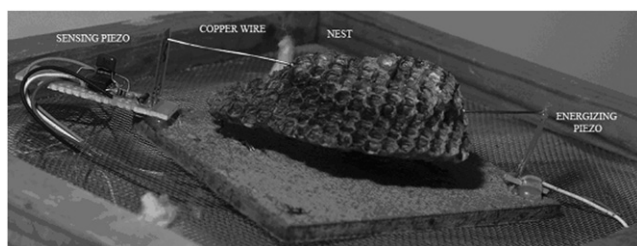


Figure 1. Experimental Set Up for a Test of the Mechanical-Switch Hypothesis [10]

The energizing piezo device on the right transmitted impulses to the nest walls at either 17 Hz or Gaussian white noise. The sensing piezo on the left measured any substrate vibrations that were generated on the nest by either artificial means or by the resident wasps. Additional components on the sensing piezo filtered out high-frequency noise coming from nearby electrical appliances and amplified recorded mechanical signals. See also related Figure S1.

significantly reduced amounts of fat, a non-diapause-related trait that corresponds to a worker phenotype [7, 8, 13]. This result is consistent with the mechanical-switch hypothesis. An important next step would be to test if the gyne-destined wasps exposed to AD-frequency pulses have an increased propensity toward worker-like behavior.

One possible mode of action of AD is via other adults, possibly by signaling them to feed less food to larvae. Three observations lead us to reject this hypothesis. First, our results show that treatment did not affect the frequencies with which adults fed the larvae. Second, if the effect of AD were indeed to decrease the amount of food fed by the adults to larvae, then the rate of development of these larvae should be much slower compared to larvae later in the colony cycle, and this is not the case. The first few workers to emerge are exposed to very high

frequencies of AD [6] yet take the least time to develop [3, 4]. Third, queens that initiate nests alone perform AD at high rates in the absence of other adults [5, 6]. These facts strongly suggest that the AD signal acts directly on the developing larvae.

Social wasp larvae sense substrate vibrations via fine hairs (mechanoreceptors) on their heads and bodies [14]. Substrate-borne mechanical signals such as AD could affect larvae by modulating the developmental pathways that underlie caste in *Polistes*. That mechanical stimuli can influence larval growth and development is well substantiated [15–17]. Mechanical stimuli are known to induce changes in the levels of biogenic amines [15–19], which in turn can influence levels of hormones with caste-influencing roles, such as juvenile hormone, in the larval stages of social insects [20], ultimately causing an epigenetic response involving differential gene expression between workers and gynes [21].

The fact that random-frequency vibrations had no significant effect on the amount of fat stores in newly emerged adults argues against AD's being a generalized mechanical stressor. Vibration at AD-typical frequency may be a signal that informs larvae of the presence of a viable egg-layer, enabling them to adopt a developmental trajectory that maximizes their inclusive fitness in the social context into which they will emerge as adults [22]. Alternatively, AD could be a manipulative signal by which egg-layers maximize their direct fitness [23]. Either way, larvae should treat AD as an honest signal because of a robustly evolved association in which those that perform AD at high rates tend to be the most socially dominant individuals on the nest [6].

The result that simulated AD vibrations had a stronger inhibitory effect on the fat stores of newly emerged adults that were third instar or younger at the start of the treatment has two important implications. First, in other eusocial Hymenoptera, including *Apis*, *Vespa*, and *Vespula*, the third larval instar is the stage at which developmental pathways of gyne- and

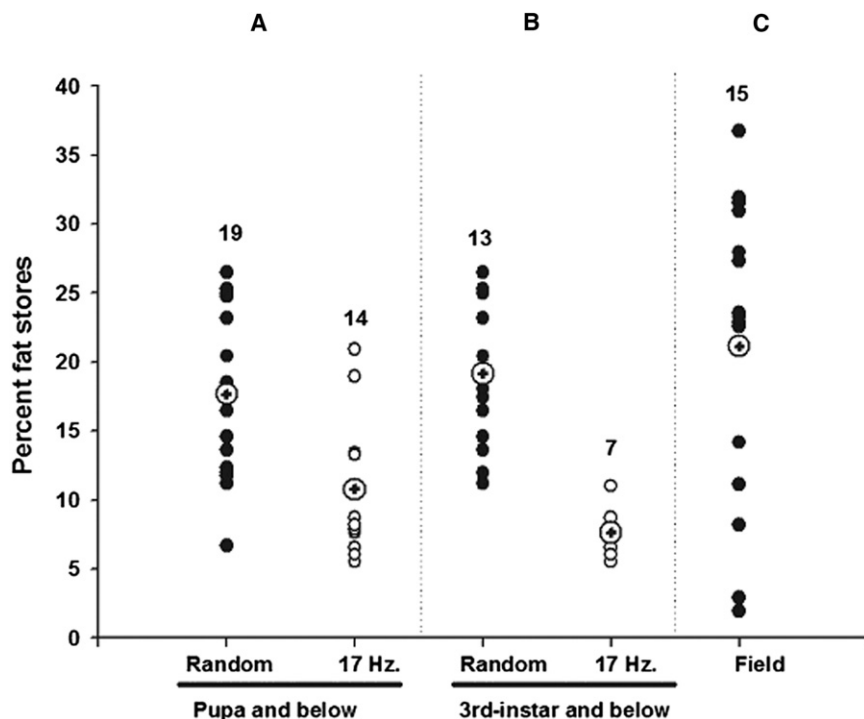


Figure 2. Newly Emerged Wasps that Had Received 17 Hz Simulated AD Impulses as Larvae Had Lower Percentage of Fat Stores Than Wasps that Received Random-Frequency Impulses

Three colonies received 17 Hz AD impulses and three others received random-frequency impulses. Each circle represents a newly emerged female wasp. Circles with cross-hairs are mean values.

(A) All wasps (n = 33).

(B) Subset of wasps that were third instar or below when piezo stimulus began (n = 20).

(C) Field wasps from three reproductive stage colonies (n = 15). Numbers above each set of circles indicate sample sizes (total number of individual wasps). See also related Figure S2.

Table 1. Main Effects of Treatment and Covariates on Percentage of Fat Stores

Effect	Type III F _{1,4} Ratio	p Value
All newly emerged wasps		
Treatment	14.2	0.019
W/L	0.01	0.91
Feeds	0.00	0.97
AD	3.45	0.14
Newly emerged wasps that were third instar or less at the start of treatment		
Treatment	35.8	0.004
W/L	0.15	0.72
Feeds	0.12	0.74
AD	2.2	0.21

Each row is a separate analysis. Treatment = 17 Hz versus random-frequency vibrations; W/L = colony-wide worker-to-larva ratio; Feeds = colony-wide frequency of prey-liquid feeding; AD = colony-wide frequency of antennal drumming.

worker-destined larvae begin to diverge [24, 25]. Coupled with the fact that AD is first seen in a colony when the oldest larvae reach the third instar [6], this suggests that the third instar is a critical stage for AD-induced caste divergence in *Polistes*. Second, the weaker effect of treatment on the larger sample, which included fourth and fifth instars and pupae, in conjunction with our result that treatment had no discernible effect on pupae, suggests that the fourth and fifth instar stages are already biased toward one or the other developmental trajectory.

It is curious that the simulated-AD-frequency group of wasps had dry weights and morphological indices of body size equal to those in the control colonies, yet emerged with significantly less fat. Because both groups had similar nutritional inputs, the possibility that in the simulated-AD group those resources were diverted to tissues other than fat should not be overlooked [26]. An alternative explanation could be that differences in the percentage of fat stores were not sufficient to translate into statistically significant differences in whole-body size and dry weight. Body size is a complex trait that is likely regulated by physiological mechanisms other than just the amount of fat stores [27]. Although neither body size nor weight is a reliable predictor of caste in *Polistes*, diapause potential is [7, 8]. How AD reduces diapause potential and the extent to which this is dependent on or independent of nutrition-induced influences on body size and weight need further investigation.

The results of our study call for a model of caste biasing in *Polistes* that incorporates both mechanical signaling and

nutrition. The first few offspring to emerge as adults not only take the least time to develop as larvae but also have small body size and negligible diapause potential, i.e., a worker phenotype [3, 4]. Their rapid development results from being fed at high levels throughout their larval period [6, 28, 29]. This is a pattern that a model based solely on differential amounts of food [2] is unable to explain: brood reared with high levels of nutrition develop rapidly, have large adult body sizes, and a high diapause potential, i.e., a gyne phenotype. Others [29, 30] have speculated about the existence of some additional ‘manipulation’ by the queen in order to explain this complex pattern. Based on the temporal pattern of AD across the colony cycle [6] and our results in this study, we believe AD to be that manipulation. Our results suggest that the high level of AD the first few offspring experience as larvae [6] inhibits their physiological capacity for diapause, as evidenced by their reduced fat stores. This not only biases development toward a worker phenotype [7], but also reduces body size and weight, which are loosely correlated with fat stores [13]. Later in the colony cycle, worker-reared larvae are fed at high rates, comparable to the first few queen-reared larvae, but receive very low levels of AD [6]. The combination of high nutrition and low AD inputs biases worker-reared offspring to develop rapidly into gynes with large body sizes and high diapause potentials. Although this model does not discount the involvement of other environmental inputs, such as differences in food *quality*, it better accounts for the complex patterns of size, development rate, and caste seen in *Polistes* than does food quantity alone.

Our findings expand our understanding of the roles played by mechanical signals in establishing and maintaining dominance among members of eusocial animal societies. Mechanical signals such as biting of brood, antennal boxing among adults in some ants, vibration signals in honey bees, and shoving in naked mole rats affect the reproductive success of recipients via direct contact with the performer [31–34]. Seen from this perspective, substrate-borne mechanical signals extend the physical (and evolutionary) terrain over which dominance interactions occur beyond direct contact and combat. Although substrate-borne vibration is a very widespread channel of animal communication [35], we believe the case reported here to be the first direct evidence of a species using substrate vibrations as a signal to modulate the development of a conspecific. Mechanoreception is one of the most ancient sensory abilities present in all invertebrate lineages [36]. Given that *Polistes* is a relatively old eusocial taxon, vibrational signaling may have been one of the earliest steps in the evolution of caste determining mechanisms in the social insects.

Table 2. Per Colony and Treatment Total Values (Mean $\pm$ SE) of Dry Weights, Percentage of Fat Stores, Antennal Drumming Rates, Prey-Liquid Feed Rates, and Worker-to-Larva Ratios

Colony	Treatment	All Wasps						Third Instar and Below	
		No. of Wasps	AD per Hr	Feeds per Hr	Worker-to-Larva Ratio	Dry Weight (mg)	Percentage of Fat Stores	No. of Wasps	Percentage of Fat Stores
1	Random	1	8.25	34.0	1.13	68.2	26.51	1	26.51
2	Random	5	4.63	29.8	0.71	46.2 $\pm$ 3.21	17.7 $\pm$ 2.12	4	19.2 $\pm$ 1.94
3	Random	13	8.75	26.0	0.61	45.1 $\pm$ 2.94	17.0 $\pm$ 1.73	8	18.2 $\pm$ 2.12
Total	Random	19	7.21 $\pm$ 1.3	29.9 $\pm$ 2.31	0.82 $\pm$ 0.16	46.6 $\pm$ 2.45	17.7 $\pm$ 1.37	13	19.2 $\pm$ 1.52
4	17 Hz	6	6.00	27.9	0.82	41.6 $\pm$ 3.26	11.0 $\pm$ 2.00	2	6.0 $\pm$ 0.52
5	17 Hz	4	2.41	34.0	1.04	42.6 $\pm$ 2.41	7.6 $\pm$ 0.56	4	7.6 $\pm$ 0.57
6	17 Hz	4	5.00	23.3	0.50	51.3 $\pm$ 6.71	13.4 $\pm$ 2.73	1	11.01
Total	17 Hz	14	4.47 $\pm$ 1.07	28.4 $\pm$ 3.11	0.75 $\pm$ 0.16	44.7 $\pm$ 2.55	10.7 $\pm$ 1.24	7	7.63 $\pm$ 0.71

Experimental Procedures

Caste-Related Measure

We chose to analyze the amount of fat stores, a diapause trait [8], because it is one of the most robust physiological measures of *Polistes* caste [7, 13], especially when assessing females from late-season colonies [7]. On such late-season nests, females with high fat stores correspond strongly to gyne behavioral profiles, whereas those with low fat stores correlate strongly with worker behavioral profiles [7]. Because postemergence intake of nutrition might alter fat stores accumulated during preadult development, we analyzed only newly emerged (≤ 1 -day-old) adult females. Because gynes and workers are not only behaviorally indistinguishable at this early adult stage [37] but also have no wing wear or ovary development [38], fat stores were the most reliable indicator of caste in this study.

Lab Wasps

Six *P. fuscatus* colonies were collected on July 9, 2007, from the UW-Arboretum, Madison, WI (43.1°N, 89.4°W) and brought into the laboratory. Colonies were collected between 0200 and 0400 hr when low temperature kept the wasps inactive, minimized disturbance, and ensured that all adults were present at the time of collection. All colonies were producing adult males and were therefore considered to be in the gyne-producing phase of the colony cycle [7, 8].

We recorded the stage of brood in each cell of each nest. Larvae were categorized to instar by head-capsule width. Nests were then placed individually in Lucite cages (20 × 20 × 20 cm) and housed under conditions of controlled temperature (25°C–27°C) and humidity (65%–75%) [39]. The photoperiod was kept at 13 hr light:11 hr dark to match ambient photophase hours in late July–August (Astronomical Applications Department, U.S. Naval Observatory), even though diapause pathways, which underlie the basis of caste in *Polistes*, are thought not to be influenced by ambient variables such as temperature or photoperiod in this taxon [8]. Each colony was provided with a daily diet of five late-instar wax-moth larvae, five mealworms, water, and honey between July 9 and September 5, 2007. Because this quantity of prey and honey was almost never completely consumed, we considered it to be *ad libitum*. All adult wasps were paint-marked on the thorax to distinguish them from subsequently emerging (unmarked) females and males, which we began to collect on the 10th day following the start of piezo treatments (see [Emerging Adults](#) below).

Field Wasps

Three gyne-phase *P. fuscatus* field colonies were collected and frozen intact with adults and brood on July 20, 2007. Only newly emerged (with black compound eyes [38]) females were used for analysis of fat stores. It is likely that the greater variability in fat stores of wasps in field colonies is due to a greater variability in the levels of available nutrition than in the lab colonies. (Lab-maintained colonies had access to uniformly high levels of nutrition.)

Piezoelectric Devices

Nest excitation was induced via a Measurement Specialties piezoelectric PVDF polymer (part no. 0-1002794-1). An 18 gauge copper wire, attached with hot-melt glue, transferred the motion of the energizing piezo (actuator) to the nest ([Figure 1](#)). The actuator piezo was programmed in LabVIEW to one of two excitation modes. The first mode (treatment) provided a 2-s-duration square wave at 17 Hz repeated every minute for 10 min for every hour during lights-on. The second mode (control) paralleled the first except that the 17 Hz square wave was replaced by a uniform white-noise signal. There were three nests in each of the two groups. That both artificial mechanical signals were transmitted through the entire nest was ascertained by measurements obtained by the acquiring piezo placed on the side of the nest opposite to the actuator piezo. The voltage output response (magnitude of nest displacement) was the measure of signal strength. We first measured the nest displacement caused by natural AD, then set the strength of the artificial AD to match it. In other words, the magnitude of the displacement of the nest (voltage response) at the point of connection of the measuring piezo was equal between the actuator-induced 17 Hz vibration and natural AD, and the corresponding power spectral density (V^2Hz^{-1}) was similar. The uniform white noise was verified by calculating the resulting power spectral density and verifying that it was constant up to the Nyquist frequency, 500 Hz. Further details are given in the [Supplemental Information](#).

By using random-frequency vibration as a disturbance control, as opposed to a no-vibration control, we could simultaneously test (1) whether AD-frequency vibrations have an effect on development, and, if so, (2)

whether the effect is specific to the AD frequency of 17 Hz and is not caused by vibration of any frequency.

Emerging Adults

All newly emerged adults (identified by the absence of paint marks) were frozen within a day of their emergence for subsequent fat analysis. Collection and freezing of newly emerged adults began on the 10th day following the start of piezo treatments. As 10 days is the minimal period of time for a pupating larva to emerge as an adult [12], this maximized the likelihood that collected adults were fifth-instar larvae or younger when piezo treatments began.

Videotaping and Analysis

Each lab colony was videotaped (Sony TRV950) once each week for a randomly selected 2 hr time period between 10 a.m. and 2 p.m., the hours of greatest activity [6]. Taping began with the start of the piezo treatments and continued for four weeks (a total of 48 hr for all the colonies). Colony-wide rates of occurrences of AD and adult feeding to larvae were determined from the videotapes.

Fat Stores

Frozen wasps were dried in a vacuum oven at 60°C and –70 kPa. Preliminary measurements showed that 4 days in the oven was sufficient to dry the frozen wasps to constant weight. All specimens were individually weighed to the nearest 0.1 mg with a Cahn 29 electronic balance. Lipids were then extracted individually from each wasp with 6 mL aliquots of chloroform-methanol (2:1 v/v) mixture on a mechanical shaker for 24 hr at room temperature (approximately 25°C). The extraction procedure was repeated three more times, each with fresh solvent aliquots, after which the wasps were redried, weighed, extracted a fifth time for 24 hr, dried and weighed again. Fourth and fifth postextraction weights did not differ significantly from each other (Mann-Whitney test, $p > 0.05$), confirming that all lipid was successfully extracted from all wasps. In individual cases where the fourth and fifth postextraction weights differed, final weight was estimated by averaging the two postextraction weights. Lipid weight was calculated by subtracting final postextraction dry weight from the pre-extraction dry weight [40]. The percentage of loss in weight (which equals the percentage of fat stores) was calculated for each individual.

Statistics

Because all the collected wasps were newly emerged from late-season nests, the amount of fat stores could be used as a reliable measure of caste [7, 13]. A mixed model analysis of variance was used to test the fixed effects of vibration treatments, feeding rates, worker-to-larva ratios, colony AD rates, and their interactions on percentage of fat stores in newly emerged adults (Proc Mixed in the SAS statistical language). Percentage of fat store data were arcsine transformed to meet normality requirements (homogeneous variance). Nest-to-nest variation was accounted for by modeling colony as a random effect. Given that there were only six colonies (and hence five degrees of freedom) for use in the analysis, it was not possible to do a single analysis examining all combinations of treatment and other colony-wide factors. Instead, we performed three different analyses:

- (1) We fitted the response to vibration treatment and each of the other three factors separately, resulting in four models, each with four degrees of freedom for error ([Table 1](#)).
- (2) From these, because only the artificial vibration treatment was important, we used a prediction model that included the vibration treatment, every other factor (separately), and their interaction. This resulted in three models, each with two degrees of freedom for error.
- (3) From these, because interactions were nonsignificant, we employed backward elimination and tested the vibration treatment with each other factor (separately) without the interaction. This resulted in three models, each with 3 degrees of freedom for error. Multiple comparisons were controlled at an experiment-wise error rate of 5% with the Tukey-Kramer method. The effects of treatment, other factors, and interactions were assessed at the 95% significance level.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at doi:10.1016/j.cub.2011.01.003.

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References

- Oster, G.F., and Wilson, E.O. (1978). *Caste and Ecology in the Social Insects* (Princeton, NJ: Princeton University Press).
- Hunt, J.H., and Amdam, G.V. (2005). Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* 308, 264–267.
- Klahn, J.E. (1981). Alternative reproductive tactics of single foundresses of a social wasp, *Polistes fuscatus*. PhD thesis, University of Iowa, Iowa City, IA.
- Miyano, S. (1983). Number of offspring and seasonal changes of their body weight in a paper wasp, *Polistes chinensis antennalis* Pérez (Hymenoptera: Vespidae), with reference to male production by workers. *Res. Popul. Ecol. (Kyoto)* 25, 198–209.
- Pratte, M., and Jeanne, R.L. (1984). Antennal drumming behavior in *Polistes* wasps. *Z. Tierpsychol.* 66, 177–188.
- Suryanarayanan, S., Hantschel, A., Torres, C.G., and Jeanne, R.L. (2010). Changes in the temporal pattern of antennal drumming behavior across the *Polistes fuscatus* colony cycle (Hymenoptera, Vespidae). *Insectes Soc.*, in press. Published online, October 08, 2010. 10.1007/s00040-010-0122-1.
- Toth, A.L., Bilof, K.B.J., Henshaw, M.T., Hunt, J.H., and Robinson, G.E. (2009). Lipid stores, ovary development, and brain gene expression in *Polistes metricus* females. *Insectes Soc.* 56, 77–84.
- Hunt, J.H., Kensing, B.J., Kossuth, J.A., Henshaw, M.T., Norberg, K., Wolschin, F., and Amdam, G.V. (2007). A diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route to caste-containing insect societies. *Proc. Natl. Acad. Sci. USA* 104, 14020–14025.
- Brillet, C., Tian-Chansky, S.S., and Le Conte, Y. (1999). Abdominal waggings and variation of their rate of occurrence in the social wasp, *Polistes dominulus* Christ. I. Quantitative analysis. *J. Insect Behav.* 12, 665–686.
- Jeanne, R.L. (2009). Vibrational signals in social wasps: A role in caste determination? In *Organization of Insect Societies*, J. Gadau and J. Fewell, eds. (Cambridge, Massachusetts: Harvard University Press), pp. 241–263.
- Gadagkar, R., Vinutha, C., Shanubhogue, A., and Gore, A.P. (1988). Preimaginal biasing of caste in a primitively eusocial insect. *Proc. R. Soc. Lond. B Biol. Sci.* 233, 175–189.
- West-Eberhard, M.J. (1969). The social biology of polistine wasps. *Misc. Publ. Mus. Zool. Univ. Mich.* 140 (Ann Arbor, MI: Museum of Zoology, University of Michigan) 1–101.
- Eickwort, K. (1969). Separation of the castes of *Polistes exclamans* and notes on its biology (Hym.: Vespidae). *Insectes Soc.* 16, 67–72.
- Yamane, S., and Okazawa, T. (1981). Mature larvae of some polistine wasps from Papua New Guinea and Fiji, with notes on larval characters of the Old World and Oceanian Polistinae (Hymenoptera: Vespidae). *Rep. Fac. Sci. Kagoshima Univ. Earth Sci. Biol.* 14, 65–75.
- Hirashima, A., Ueno, R., and Eto, M. (1992). Effects of various stressors of larval growth and whole-body octopamine levels of *Tribolium castaneum*. *Pestic. Biochem. Physiol.* 44, 217–225.
- Hirashima, A., Nagano, T., and Eto, M. (1993). Stress-induced changes in the biogenic amine levels and larval growth of *Tribolium castaneum* Herbst. *Biosci. Biotechnol. Biochem.* 57, 2085–2089.
- Hirashima, A., Takeya, R., Taniguchi, E., and Eto, M. (1995). Metamorphosis, activity of juvenile-hormone esterase and alteration of ecdysteroid titres: Effects of larval density and various stress on the red flour beetle, *Tribolium freemani* Hinton (Coleoptera: Tenebrionidae). *J. Insect Physiol.* 41, 383–388.
- Davenport, A.K., and Evans, P.D. (1984). Stress-induced changes in octopamine levels of insect haemolymph. *Insect Biochem.* 14, 135–143.
- Rauschenbach, I.Y., Serova, L.I., Timochina, I.S., Chentsova, N.A., and Schumnaja, L.V. (1993). Analysis of differences in dopamine content between two lines of *Drosophila virilis* in response to heat stress. *J. Insect Physiol.* 39, 761–767.
- Rachinsky, A. (1994). Octopamine and serotonin influence on corpora allata activity in honey bee (*Apis mellifera*) larvae. *J. Insect Physiol.* 40, 549–554.
- Toth, A.L., Varala, K., Newman, T.C., Miguez, F.E., Hutchison, S.K., Willoughby, D.A., Simons, J.F., Egholm, M., Hunt, J.H., Hudson, M.E., and Robinson, G.E. (2007). Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science* 318, 441–444.
- Liebig, J., Monnin, T., and Turillazzi, S. (2005). Direct assessment of queen quality and lack of worker suppression in a paper wasp. *Proc. Biol. Sci.* 272, 1339–1344.
- Alexander, R.D. (1974). The evolution of social behavior. *Annu. Rev. Ecol. Syst.* 5, 325–383.
- Ishay, J. (1975). Caste determination by social wasps: Cell size and building behavior. *Anim. Behav.* 23, 425–431.
- Evans, J.D., and Wheeler, D.E. (1999). Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. *Proc. Natl. Acad. Sci. USA* 96, 5575–5580.
- Rubin, C.T., Capilla, E., Luu, Y.K., Busa, B., Crawford, H., Nolan, D.J., Mittal, V., Rosen, C.J., Pessin, J.E., and Judex, S. (2007). Adipogenesis is inhibited by brief, daily exposure to high-frequency, extremely low-magnitude mechanical signals. *Proc. Natl. Acad. Sci. USA* 104, 17879–17884.
- Nijhout, H.F. (2003). The control of body size in insects. *Dev. Biol.* 261, 1–9.
- Mead, F., Habersetzer, C., Gabouriaux, D., and Gervet, J. (1994). Dynamics of colony development in the paper wasp *Polistes dominulus* Christ (Hymenoptera, Vespidae): The influence of prey availability. *J. Ethol.* 12, 43–51.
- Kudo, K. (2003). Growth rate and body weight of foundress-reared offspring in a paper wasp, *Polistes chinensis* (Hymenoptera, Vespidae): No influence of food quantity on the first offspring. *Insectes Soc.* 50, 77–81.
- Miyano, S. (1998). Amount of flesh food influences the number, larval duration and body size of first brood workers, in a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). *Entomol. Sci.* 1, 545–549.
- Brian, M.V. (1973). Caste control through worker attack in the ant *Myrmica*. *Insectes Soc.* 20, 87–102.
- Monnin, T., and Peeters, C. (1999). Dominance hierarchy and reproductive conflicts among the subordinates in a monogynous queenless ant. *Behav. Ecol.* 10, 323–332.
- Boucher, M., and Schneider, S.S. (2009). Communication signals used in worker-drone interactions in the honey bee, *Apis mellifera*. *Anim. Behav.* 78, 247–254.
- Reeve, H.K. (1992). Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature* 358, 147–149.
- Hill, P.S.M. (2008). *Vibrational Communication in Animals* (Cambridge, MA: Harvard University Press).
- Desutter-Grandcolas, L., Blanchet, E., Robillard, T., Magal, C., Vannier, F., and Dangles, O. (2010). Evolution of the cercal sensory system in a tropical cricket clade (Orthoptera: Grylloidea: Eneopterinae): A phylogenetic approach. *Biol. J. Linn. Soc. Lond.* 99, 614–631.
- Giray, T., Giovanetti, M., and West-Eberhard, M.J. (2005). Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci. USA* 102, 3330–3335.
- Haggard, C.M., and Gamboa, G.J. (1980). Seasonal variation in body size and reproductive condition of a paper wasp *Polistes metricus* Hymenoptera Vespidae. *Can. Entomol.* 112, 239–248.
- Suryanarayanan, S., and Jeanne, R.L. (2008). Antennal drumming, trophallaxis, and colony development in the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). *Ethology* 114, 1201–1209.
- O'Donnell, S., and Jeanne, R.L. (1995). Worker lipid stores decrease with outside-nest task performance in wasps: Implications for the evolution of age polyethism. *Experientia* 51, 749–752.