

REPRODUCTIVE CASTE DETERMINATION IN EUSOCIAL WASPS (HYMENOPTERA: VESPIDAE)

Sean O'Donnell

Department of Psychology, Box 351525, University of Washington, Seattle,
Washington 98915; e-mail: sodonnel@u.washington.edu

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ABSTRACT

Wasps (Vespidae) exhibit a range of social complexity, from solitary living to eusocial colonies, and thus are exemplary for studies of the evolutionary origin and maintenance of social behavior in animals. Integral to the definition of eusociality is the presence of reproductive castes, group members that differ qualitatively in their ability to reproduce in a social setting. Behavioral and morphological evidence suggests that caste determination, the developmental process by which differences in fecundity are established, occurs to a large extent before adult emergence (pre-imaginally) in many species of Vespidae, in both basal and advanced taxa within the clade (Vespinae + Polistinae), which includes most eusocial species. Pre-imaginal determination has been documented in many taxa (e.g. independent-founding Polistinae) where it was not thought to occur. Correlative and experimental studies indicate that differences in nutrition during larval development are often the basis of pre-imaginal caste determination. Pre-imaginal caste determination has important implications for the roles of subfertility and manipulation by nest mates in the evolution of eusocial behavior.

PERSPECTIVES AND OVERVIEW

The generally accepted definition of eusociality (69, 135) was proposed by Batra (7) and includes three criteria for animal societies: overlap of adult generations in the group, cooperation in brood care, and reproductive division of labor. Because it involves individuals foregoing personal reproduction to aid others, the last attribute appears to run counter to the expected outcome of evolution by natural selection, and it has therefore been the focus of much interest from

evolutionary biologists. Several recent reviews on the evolution of eusocial behavior are available (2, 3, 59, 115). Whether sterility on the part of helpers (workers) must be permanent and obligate or temporary and facultative is left ambiguous in the above definition, and controversy over what criteria should be used to delimit eusociality has arisen. Knowledge of the mechanisms and developmental timing of differentiation among individuals with reproductive strategies is therefore critical to the definition of eusociality and to understanding the evolution of eusocial behavior (29, 57, 131).

Wasps of the family Vespidae have been important subjects in efforts to unravel the patterns and processes of the evolution of eusocial behavior, as extant species of vespids exhibit grades of sociality ranging from solitary nesting to eusocial behavior (19, 49). The systematics of the Vespidae has recently been revised by Carpenter (15, 16).

In addition to reproductive division of labor, which is the topic of this review, "division of labor" applies to separation of tasks within the worker force (polyethism), which is not covered here. Mechanisms of division of labor among social insect workers have recently been reviewed (93), and polyethism in Vespidae was reviewed by Jeanne (50). I do not consider the evolution of socially parasitic species (79), which by definition lack worker castes.

Although I address the physiological basis of caste determination from an ecological perspective, I do not treat the endocrinological aspects of caste differences. Reviews of the physiological mechanisms of caste determination can be found in References 23, 24, 29, 107, 133.

In some species of Vespidae, females of the same generation (cofoundresses) cooperate in initiating new colonies. Since the pioneering observations of Pardi (83), patterns of cofoundress interactions and the mechanisms of reproductive differentiation among foundresses have received a great deal of attention (78, 86, 122). Behavioral interactions among adult females of the same generation can enhance differences in reproductive potential and lead to division of labor. These interactions are of interest in their own right because they reveal a great deal about the pursuit of alternative strategies by different phenotypes within populations (132). Although eusociality may have arisen in the context of social interactions among females of the same generation (129, but see 14), foundress interactions may not tell us anything about caste determination leading to eusociality, that is, the development of a set of sterile cooperative offspring. In his recent reviews of the role of nutrition in the evolution of caste in the Vespidae, Hunt (43, 44) made a clear distinction between offspring, which he termed ontogenic workers, and cooperative helpers from the same generation, termed subordinate workers.

In the literature on reproductive castes in Vespidae, it is often stated that morphological castes are rare or nonexistent outside the Vespinae (hornets and

yellow jackets) and, therefore, that the concept of caste (e.g. grouping females into discrete categories of queens and workers) cannot be applied to most eusocial Vespidae (57, 107, 133). In this review I present evidence that these statements are incorrect. My aim is to review studies that address the developmental timing and mechanisms of caste determination in the Vespidae.

INTRODUCTION

As currently defined, the hymenopteran family Vespidae comprises five subfamilies and includes species spanning all grades of social behavior, from solitary to advanced eusocial (swarm-founding) (15, 16). Because of their diversity of social structure, vespid wasps have long been recognized as a key group to understanding the evolutionary origins and maintenance of reproductive division of labor, wherein some individuals reproduce while others forego reproduction and help raise offspring (27, 135). Eusocial behavior in the Vespidae is exhibited in the clade comprising the subfamilies Stenogastrinae, Vespinae, and Polistinae. Some eusocial species are independent founders: Females of one generation, either alone or in cooperative groups, initiate new nests. Others are swarm-founders: Females from more than one generation move to new sites in a coordinated group and initiate nests (49).

Excepting the subfamily Vespinae, many studies of wasp reproductive division of labor have assumed that interactions among adult females are the most important or even the only factor in determining which individuals reproduce in eusocial Vespidae (12, 107, 133, 135). Some authors even suggest that the term reproductive caste cannot be applied to vespid wasps other than the Vespinae (107). I refer to caste determination that occurs after adult emergence, often as a result of social interactions (21), as imaginal determination.

Adult interactions influence the favored reproductive strategies of females in some wasp societies. In some vespid species, caste status is flexible for at least some individuals, and in extreme cases no females may be fully excluded from reproducing (some Stenogastrinae: 118, 119; *Ropalidia rufoplagiata*: 101). For other wasp species, we must address the question of whether all eclosing females have the potential to reproduce. Several lines of evidence suggest that the answer may often be “no.” In a diversity of eusocial wasp lineages, reproductive castes differentiate during immature development (i.e. before adult emergence). I refer to caste determination that occurs before adult emergence, principally during larval development, as pre-imaginal determination.

In this review I summarize evidence for the occurrence of pre-imaginal and imaginal caste differentiation in eusocial Vespidae. Empirical evidence for pre-imaginal differentiation is of two types. First, members of different castes can sometimes be distinguished by discrete differences in body shape and/or

size that correspond to qualitative differences in reproductive potential (morphological castes). More species of vespid wasps exhibit morphological caste differences than is generally acknowledged, and new evidence for morphological caste differences is accumulating in a number of taxa. Second, recent studies suggest that pre-imaginal caste differentiation need not be reflected by differences in external morphology among castes. Females of monomorphic species can differ qualitatively in their ability to reproduce independently of social interactions or environmental variation during adulthood. These disparities in reproductive ability are established prior to adult eclosion, probably in response to differential larval nutrition. Phylogenetic evidence suggests that pre-imaginal differentiation of wasp reproductive castes is a general rather than an exceptional pattern among the eusocial Vespidae.

DEFINITIONS OF CASTE

Previously Proposed Definitions

Some authors propose that developmentally fixed sterility should not be a criterion for eusociality, such that species with temporary helpers that may later reproduce are considered eusocial (31, 55, 99). Under this definition, permanent differentiation of castes is not required of eusocial species, although helping behavior is required.

Other authors propose the opposite approach, requiring that permanent obligate sterility of helping individuals be used as the hallmark of eusociality (21, 116). The modified term highly eusocial was proposed by Michener (70) to denote a distinct evolutionary grade of social behavior for those species characterized by morphological reproductive caste differences. This view has been extended by Villet (124), who argued that the term caste be retained solely for morphologically distinct individuals that are the outcome of discrete developmental trajectories (see also 21, 84a). Following this definition, morphologically distinct individuals belong to different castes regardless of their social function (role) in the colony. This viewpoint relies in part on the assumption that morphological differences are unique indicators of distinct developmental pathways leading to castes. This assumption may not be correct, however, given mounting evidence for pre-imaginal caste determination that is not reflected in (external) morphology.

The Definition of Caste as Used in this Review

The focus of this review is on the mechanisms that underlie the dramatic differences in life history among queens and workers. I use the term caste in reference to female reproductive function, with the requirement that caste membership be developmentally determined and irreversible (116). Continuous phenotypic variation in response to variation in environmental factors during development

can be classified as a reaction norm, whereas discontinuous phenotypic variation is referred to as a polyphenism (134). I propose that the term caste should be applied to reproductive polyphenism among individual social insects. However, this polyphenism need not be reflected in external morphology, and morphology will not be used as the main criterion for delimiting castes.

Complete loss of direct reproductive function in females is restricted to some species of ants, where workers lack ovaries (39). Even in species where preimaginal caste determination is unquestioned, worker Vespidae are generally not completely sterile (11). As in highly eusocial bees (136), workers of many vespid species can and do lay eggs, especially when the queen is lost or removed (even in Vespinae: 58). I propose that the critical issue to delimiting castes is whether all adult females can be inseminated and lay fertile eggs, as discussed below.

Using terminology from developmental biology, I refer to the processes leading to the establishment of caste differences as caste determination, which can take place before or after adult eclosion. Caste differentiation is the permanent assignment of females to the queen caste (within which there can still be variability in reproductive potential) or to the worker caste (either sterile or restricted to production of males). Note that Brian (12) reversed the meanings of the terms determination and differentiation in discussing caste.

HOW CASTES ARE DISTINGUISHED

Behavioral Roles

Various behavioral differences among females have been employed to distinguish caste status of female Vespidae. The most obvious behavioral role of queens is reproduction, i.e. egg-laying (60). The observation of multiple egg-layers may suggest the presence of more than one queen (polygyny: 47). However, other females often lay eggs in wasp societies, even when the queen is present, and egg-laying does not always indicate that a female has been inseminated. In some species, queens are more likely to eat the eggs laid by other females, and differential oophagy can be used as a behavioral indicator of caste (127).

Within colonies, workers may be more likely to perform such risky tasks as nest defense (36) and foraging (142), although interspecific comparison showed that differences in task performance are not always indicative of caste differences (80). The ability to found new nests has also been used to identify queens (33, 107).

Reproductive Physiology

INSEMINATION Numerous studies have employed insemination as the main criterion for distinguishing queens from workers (38, 87, 88, 92), and it is the criterion advocated here. Incorrect assignment of caste for some females may

result when employing this method because young (recently eclosed) gynes may be present in a collection that have not yet copulated and whose ovaries have yet to develop. Ovary development can precede insemination in *Polistes erythrocephalus* queens (127); it is not known whether this pattern is common in the Vespidae, particularly in relatively aseasonal tropical habitats.

Insemination can be assessed readily in freshly collected specimens by examining the spermatheca (sperm-storage organ) under magnification. If it contains sperm, the center of the spermatheca appears opaque and opalescent. Determining insemination status in fixed material is more difficult but still possible. Reed et al (88) squashed fixed spermathecae on microscope slides and stained them with methylene blue; sperm flagella were visible in inseminated females under a compound light microscope at 1000X. Carpenter & Ross (17) were able to identify full spermathecae in fixed material with phase-contrast microscopy.

OVARY DEVELOPMENT Unless they can be examined immediately, field-collected specimens should be fixed, for example in Kahle's or Dietrich's solution (6), and later transferred to 70–80% ethanol for storage. Ovaries can shrink or distort when specimens are immediately stored in ethanol (G Eickwort, personal communication). Many studies of caste status have relied on ovary development alone to distinguish queens from workers. Agreement between ovary development and insemination is generally good in some species (92, 121), but if the criterion for caste assignment involves ability to produce fertile offspring, then insemination status should be used whenever possible. Even in species with discrete morphological caste differences, workers with developed ovaries are sometimes collected from queenright colonies (121, 125). The existence of said workers does not necessarily imply caste flexibility; whether laying workers are inseminated or can produce fertile eggs is rarely ascertained.

FAT BODY Eickwort (25) reported qualitative differences in the amount and morphology of the fat body in female *Polistes exclamans* emerging in late summer. Fat body differences corresponded to behavioral differences and have since been used as an indirect indicator of caste status of temperate-zone independent-founding wasps (38, 60, 108).

EVIDENCE FOR PRE-IMAGINAL DETERMINATION OF CASTE

Morphological Caste Differences

To date, most evidence for pre-imaginal caste determination derives from studies that correlate external cuticular morphology with behavioral or physiological differences (e.g. ovary development or presence of sperm in the spermatheca).

Once an insect emerges to the adult (reproductive) stage, it is enclosed in a solid nonregenerating cuticle. Other than damage and wear, further morphological change of hardened (sclerotized) body parts is impossible, and any differences in cuticular morphology among adults are the result of developmental processes during the immature stages. External morphological differences that correlate with reproductive caste are convenient and clear indicators that reproductive castes differentiate before adulthood.

Some eusocial wasps exhibit isometric growth and have a unimodal body-size distribution among females. In these species, size relative to nest mates generally plays an important role in imaginal caste determination, but size is not an absolute indicator of reproductive potential (18, 25, 60, 122; but see 109). Therefore, nonisometric growth (i.e. growth leading to differences in body shape) provides the clearest evidence for morphological caste differences (9, 77, 124). However, bimodal size distributions among reproductives and nonreproductives can also be considered as evidence for morphological castes in isometric species (134, 135).

The Vespinae are sometimes considered to be the only highly eusocial vespid wasps because their queen/worker dimorphism is well developed (65). However, many other vespids exhibit discrete morphological caste differences. These queen/worker differences are usually more subtle than those in the Vespinae. The structures that are compared between queens and workers must be chosen carefully, as not all body parts will indicate deviations from isometry. In many eusocial wasps, allometric caste differences are most pronounced in the structures of the gaster (9, 52, 64, 121).

In Neotropical swarm-founding wasps of the tribe Epiponini, a number of species have morphologically distinct queens, based on size measurements and dissections. Species in several epiponine genera exhibit dimorphism (Table 1). The set of genera known to exhibit caste polymorphism include the most basal and the most derived epiponines (126). In particular, some species of *Agelaia* have well-developed caste allometry with bimodal body size distributions and differences in shape similar to Vespinae (28, 52; S O'Donnell, personal observation). In some species queens are actually smaller than workers, and, in others, queens are smaller anteriorly (head measurements) and larger posteriorly (gaster measurements) (49, 64). Pre-imaginal differentiation is therefore widespread, if not characteristic, in this tribe. Studies purporting to refute the existence of morphological castes should be viewed with caution, especially if gastral characters were not measured (110).

Morphological caste differences have also been recorded in the Ropalidiini, a polistine tribe comprising four Old-World genera (16; Table 1). Relative to other polistines, ropalidiines are poorly studied but are known to include independent-founding species (*Belonogaster*, *Parapolybia*, and some *Ropalidia*

Table 1 Studies reporting queen/worker dimorphism in Polistinae

Tribe: Species (Ref.)	Size difference ^a	Gaster difference ^b	Color difference	Sample size ^c		
				Number queens	Number workers	Number colonies
Ropalidiini						
<i>Polybioides raphigastra</i> (30)	N	Y	Y	nr	nr	nr
<i>Polybioides tabidus</i> (121)	Y	Y	nr	82	188	3
<i>Belonogaster grisea</i> (84)	Y	Y	nr	18	17	1
<i>Belonogaster juncea</i> (89)	Y	Y	nr	nr	nr	nr
<i>Ropalidia ignobilis</i> (125)	Y	Y	Y	4–8	49–137	18
<i>Ropalidia montana</i> (140)	Y	Y	nr	nr	nr	nr
Epiponini						
<i>Apoica flavissima</i> (100)	Y	Y	Y	5	295	1
<i>Apoica pallens</i> (53)	N	Y	nr	50	50	1
<i>Agelaia areata</i> (52)	Y	Y	Y	167	195	4
<i>Agelaia flavipennis</i> (27)	Y	Y	Y	nr	nr	nr
<i>Agelaia fulvofasciata</i> (90)	nr	Y	Y	nr	nr	nr
<i>Agelaia vicina</i> (97)	Y	Y	N	100	60	1
<i>Agelaia xanthopus</i>	Y	Y	N	nr	nr	nr
<i>Agelaia yepocapa</i>	Y	Y	Y	nr	nr	nr
<i>Pseudopolybia difficilis</i> (51)	N	Y	nr	26	165	1
<i>Protopolybia pumila</i> (92)	Y	nr	nr	20	20	nr
<i>Protopolybia minutissima</i> (92)	nr	Y	nr	12	12	5
<i>Brachygastra lecheguana</i> (64)	N	Y	nr	50	50	1
<i>Brachygastra scutellaris</i> (92)	nr	nr	Y	108	161	2
<i>Brachygastra scutellaris</i> (17)	Y	N	Y	34	33	1
<i>Protonectarina sylveirae</i> (144)	nr	Y	nr	nr	nr	nr
<i>Polybia catillifex</i> (17)	Y	Y	nr	5	42	1
<i>Polybia dimidiata</i> (92)	Y	nr	nr	nr	nr	nr
<i>Polybia occidentalis</i>	nr	Y	nr	7	7	1
<i>occidentalis</i> (92)						
<i>Polybia occidentalis</i>	Y	Y	nr	54	234	6
<i>platycephala</i> (92)						
<i>Polybia parvula</i> (92)	nr	Y	nr	18	18	1
<i>Metapolybia cingulata</i> (92)	Y	nr	Y	2	4	1
<i>Epipona guerini</i> (46)	Y	Y	nr	50	50	1
<i>Epipona tatua</i> (91)	Y	Y	nr	30	60	nr

^aDifferences in overall body size based on wing length, total body length, or size of thoracic structures.

^bDifferences in the size and/or shape of one or more gastral segments.

^cnr = not reported.

spp.) and swarm-founding species (*Polybioides* and the remaining *Ropalidia* spp.). Wenzel (125) documented morphologically distinct queens in *Ropalidia ignobilis*, which as an independent-founding wasp was assumed to exhibit only imaginal caste determination (49). Turillazzi et al (121) and Francescato et al (30) noted discrete structural and significant size differences that corresponded almost perfectly with ovary development in the swarm-founding wasp *Polybioides tabidus* and *Polybioides raphigastra*. Similarly, Yamane et al (140) showed that queens (inseminated females with developed ovaries) of the swarm-founder *Ropalidia montana* were larger than workers in all characters measured; caste size differences increased in magnitude from head to gaster, as in many epiponines. Kojima (56) stated that queens and workers differed morphologically in swarm-founder *Ropalidia romandi* but presented no data. The degree of caste differences may vary among colonies (17, 56), and samples of queens and workers from several colonies should be analyzed separately whenever possible.

Few studies have employed powerful and sensitive morphometric techniques (9, 46, 51) to examine caste differences in morphology in independent-founding genera (141). In particular, studies are needed that employ measurements of the relative sizes and shapes of structures in all three major body sections (head, mesosoma, and metasoma) and compare them among queens and workers.

Physiological Pre-Imaginal Determination

Pre-imaginal caste differentiation may occur that is not reflected in external morphological differences. Several selective factors can limit or preclude external morphological differences between queens and workers. The degree of external morphological caste differences may be influenced by ecological factors that are independent of the system of social interactions within colonies. For example, the need to move frequently or rapidly to new nest sites in response to disturbance may constrain the evolution of morphologically distinct queens. The need for queens to overwinter in temperate climates may have selected for extreme queen/worker dimorphism in some species of Vespinae (129), and variation in queen body size has been shown to influence overwinter survival in species not known to possess morphological castes (22). Attention is increasingly paid to the role of parasites as selective factors in social insect evolution (98). Keller (54) suggested that different morphological castes within ant workers could retard the transmission of parasites within colonies. Divergent morphology and physiology among worker castes may make them differentially susceptible to some pathogens. Similarly, queen/worker dimorphism could function to restrict transmission of parasites from workers, who are presumably more likely to contact infective agents while foraging, to reproductives (81). In some eusocial wasps, contact between queens and workers is

minimal (1), which could further limit intracolony spread of pathogens. Parasites and pathogens may be a selective factor favoring pre-imaginal caste determination.

Morphologically discrete queen and worker castes clearly indicate the operation of pre-imaginal caste determination, but morphological differences are not a necessary feature of pre-imaginal caste bias. For example, females could differ physiologically or in internal morphology upon adult emergence, such that some are fully excluded from reproducing. Internal structures of queens and workers can differ dramatically; for example, the number of Malpighian tubules and length of the gut can be greater in queens than in workers in Vespidae (V Mauss, personal communication). Whether these anatomical differences correspond predictably with caste in species without external morphological differentiation remains to be examined. Recent evidence suggests that pre-imaginal caste differentiation can in fact occur without accompanying external morphological differences.

Studies by Gadagkar et al (32, 33) on *Ropalidia marginata* suggest pre-imaginal caste biasing in a species that lacks apparent morphological castes. In the first study, freshly eclosed females were isolated from 22 field-collected nests to remove the effects of social experience and were fed ad libitum as adults (33). Only approximately 50% (97 of 197) of these females built nests and laid eggs before perishing, and the time of year when parent nests were collected had no effect on the probability of nesting by emerging females. One morphological character, a measure of head size, bore a negative relationship with probability of nesting; gastral characters were not measured. In a follow-up experiment with 6 nests, a similar percentage (47 of 87) of females eventually laid eggs; the mean rate of larval feeding at a given nest bore a positive relationship with the probability of its females laying eggs. These studies are especially relevant to nutritional models of caste determination (43) because differences in food availability following adult emergence were not a factor contributing to behavioral variability.

Work on the monomorphic wasp *Polistes dominulus* (= *gallicus* of Grechka & Kipyatkov) suggests that most individuals were reproductively differentiated prior to adult emergence (35). Grechka & Kipyatkov (36) showed that *P. dominulus* females' reproductive caste, as determined by an assay of defensive behavior in response to simulated predator approach, corresponded with the number of adults present on the nest during their larval development. Wasps emerging from nests with more females were more likely to behave as nondefensive gynes. The authors concluded that more females on the nest provided more brood care, including a higher rate of larval feeding. Laboratory experiments conducted by Mead & Gabouriaux (66) support this conclusion. Ability to survive the winter was used as an indicator of caste; temperate *Polistes* spp.

workers differ physiologically from gynes such that gynes are more likely to survive cold temperatures (25, 102, 108). *P. dominulus* females eclosing from nests attended by queens alone had low probability of winter survival, whereas those eclosing from nests with workers were more likely to survive (66). Lorenzi & Turillazzi (63) noted that females belonging to discrete categories with respect to fat deposition and behavior, which they classified as workers and gynes, emerged simultaneously as the first offspring produced by *Polistes biglumus* colonies in the alpine zone.

The performance of worker-like behavior (e.g. foraging, nest defense) by an individual does not necessarily indicate that that individual is incapable of later becoming a queen (80). In at least some species, especially of independent-founding wasps, offspring females that engage in work for the colony are the most likely to assume the role of reproductives if the queen is lost (41, 71, 109). Furthermore, queens of some species engage in foraging (63). In these species, castes are not readily distinguished by task performance, except by differential rates of egg-laying, oophagy, and in some cases by the outcomes of dominance interactions. Pre-imaginal caste determination predicts that some females will not engage in reproduction, but it does not require that reproductive females avoid labor for the colony. Queen and worker behaviors do not necessarily represent complementary alternative strategies (131), and the degree of behavioral differentiation between the castes depends on the ecology and social structure of the species or population in question (80).

Social wasp larvae are evidently affected by their nutritional environment. Nixon (cited in 92) noted that when bad weather prevented adult wasps from foraging, there was visible shrinkage of the larvae in the nest. The few data available on larval behavior suggest that developing wasps respond to changes in the nutritional status of their colonies. Larvae of *Mischocyttarus immarginatus* from pre-emergence nests were more likely to yield nutritious (high amino acid and carbohydrate content: 45) saliva upon solicitation by adults than were larvae from nests with workers (42). The author proposed that rich saliva could forestall cannibalism by adults in food-stressed colonies but was probably costly to the larva's own nutritional reserves.

Combined with data from highly eusocial species, these studies indicate that differential larval nutrition may be a common mechanism in pre-imaginal caste determination. Hunt (43, 44) suggested that the worker caste may be "invented anew in every generation," with variation in larval nutrition playing a central role in female caste determination. Wheeler (133) proposed that nutritional switches channel females of highly eusocial species into discrete developmental pathways (castes). It appears that such developmental mechanisms play a role in caste determination throughout the eusocial Vespidae, in species of all grades of social complexity and all modes of colony foundation.

EVIDENCE FOR IMAGINAL DETERMINATION OF CASTE

Lack of Morphological Differences

The lack of consistent morphological differences between functional (or physiological) queens and workers is often cited as evidence against pre-imaginal caste determination. Caution must be applied when interpreting much of the negative evidence for morphological caste differences. In many if not most cases, these data have not been collected in ways that convincingly rule out the existence of morphological differences. Even when documented properly, a lack of morphological differences in and of itself does not necessarily rule out pre-imaginal determination.

Queen Succession and Experimental Queen Removal

Studies of queen succession are easily performed on independent-founding species. Once the queen is identified, she can be removed experimentally, and natural rates of queen disappearance are high in some populations (40, 48, 109). Quantification of the behavioral and morphological attributes of females that succeed the queen can yield insight into mechanisms of both pre-imaginal and imaginal caste determination. Queen succession studies should be designed such that the generation to which females belong is known. In some cases, cofoundresses remaining on the nest are likely to take over following queen loss, and offspring females do so only in their absence (40).

Several studies have documented female daughters taking over the role of queen following queen removal or natural disappearance. Replacement queens are identified as the individuals that lay the largest numbers of eggs following queen loss and are often the most dominant individuals. In *Polistes metricus*, females emerging in June developed their ovaries following queen removal, whereas females emerging late in the summer (presumably gynes about to enter diapause) did not (10). In *Polistes jadwigae* and *Polistes chinensis antennalis*, offspring females that took over as queens produced only males (73, 75). In other species, it was not determined whether the females that replaced queens produced only males (*Mischocyttarus labiatus*, 62; *P. metricus*, 10; *Polistes annularis*, 40), but daughter females that replaced queens were inseminated in *Mischocyttarus flavitarsis* (61) and thus may have produced female offspring. In this context, it is important to distinguish female offspring that do not mate (e.g. because there are no males present in the population) from those that cannot or will not mate because their role as workers has been determined developmentally (142). In *Belonogaster grisea*, slender-bodied females were more likely to behave as workers and refused to copulate with males when presented the opportunity (84), which suggests that inability to mate is

developmentally determined in at least some species of independent-founding wasps.

Solís & Strassmann (102) removed brood from some nests onto which *P. exclamans* females emerged, and they showed that cold tolerance of females on nests without brood to care for was increased relative to control females. Because it had earlier been shown that *Polistes* gynes were more cold hardy than nest-mate workers (108), the authors concluded that imaginal determination of caste had occurred, with labor-demanding brood causing worker-like physiology in the control females. However, whether and how the physiological differences measured correspond to differences in social and reproductive behavior was not determined. Mead et al (67) induced nest-founding by *P. dominulus* offspring females by removing their nests and queens and then placing them in groups in cages with nest construction materials. A subset of daughters that had behaved as workers were able to found new nests and lay (unfertilized) eggs. Variation in nest-founding ability occurred both within and among colonies. It is important to note that the colonies had been provided with ad libitum sugar and protein sources while the subject wasps were immature because unlimited food during larval development may have dramatically affected the caste status of the subjects (see “Mechanisms of Caste Determination” below).

SPECIES LACKING PERMANENT CASTES

In some social species of Polistinae, many, if not all, females forego reproduction only temporarily. In these species, helping females often have the opportunity to reproduce later in their lives (101, 118, 119), as is the case in birds with helpers at the nest (26, 99). By the definition employed here, reproductive castes are not present in these species.

Observations on a single colony of *R. rufoplagiata* suggest that division of labor may be temporal in this species (101). Many females were inseminated egglayers, and younger females performed most of the labor (e.g. foraging) for the colony, which suggests that most or all females may reproduce when older. However, the colony was supplied with ad libitum sugar and protein sources in a vespary, which may have influenced the caste status of females emerging on the nest.

The subfamily Stenogastrinae includes social species and is the sister clade to the (Polistinae + Vespinae), the clade that comprises all eusocial Vespidae (13, 15). Field studies of social Stenogastrinae suggest that most, if not all species, exhibit nest sharing with temporal patterns of division of labor among females. In many cases, only young females are not inseminated or have undeveloped ovaries, and many or all females may have at least the possibility of reproducing directly (118, 119, 143). If this generalization proves correct,

then the evolution of true worker castes must be studied in the Polistinae. However, in the stenogastrine *Parischnogaster nigricans*, fertilized females were found to have larger heads than unfertilized females (18 of 25 females from seven colonies were fertilized), indicating the possibility of developmentally determined caste (123).

Serial polygyny occurs in colonies of some independent-founding polistine wasps, apparently often as a result of daughters dominating and ejecting their mothers and then superseding them as queens (48, 139). In these species, superseding daughters can mate and produce female offspring, but whether most or all females are capable of doing so is uncertain.

Successive experimental queen removals can help establish which offspring females are capable of replacing the queen as fertile reproductives, and they complement observational studies documenting the presence of multiple inseminated females on nests. Suzuki (112) removed queens from 20 *Polistes snelleni* colonies and measured subsequent reproduction by offspring females. Offspring females succeeding the queen on 7 colonies were inseminated; because these females emerged in June and July, they were presumed to be workers.

MECHANISMS OF CASTE DETERMINATION

Nutrition During Larval Development

The opinion expressed by Pardi (83) for *Polistes gallicus* that caste determination "is independent of the nutrition received during larval life" has been very influential to vespid biologists, but it clearly needs to be reexamined. In independent-founding species, the first female offspring to emerge are often smaller than queens and cofoundresses (*Mischocyttarus*: 61; *Polistes*: 22, 72, 117; *Parapolybia*: 138), but differences in body size can disappear as the season progresses (38). Similar patterns have been observed in vespines (4, 103), including tropical species (105), and in eusocial bees (76). Even among workers, body size differences can affect dominance status and production of male eggs. In vespines, workers that lay male eggs under queenright conditions are often larger than nonlaying nest mates (94).

Seasonal increases in female body size have often been attributed to changes in the worker/larva ratio as the colony develops. When more adults are present per larva, food availability presumably increases, leading to greater size at eclosion (4, 127). The estimated total amount of protein arriving at *Polistes chinensis* colonies increased exponentially with colony size, suggesting a more favorable nutritional balance in larger post-emergence colonies (111). Litte (61) removed adult workers from *M. flavitarsis* colonies and found that, at adult eclosion, body size of the offspring decreased (relative to contemporaries on unmanipulated nests) for the individuals that were larvae when the workers

were removed. Grechka & Kipyatkov (36) noted that the appearance of early reproductives (females that did not work for their colonies and were expelled from their nests) among the first offspring occurred more frequently in *P. dominulus* colonies with several foundresses than in single-foundress colonies. The authors concluded that larvae in colonies with several foundresses were better fed.

Careful attention to variation in the timing of production of reproductives by colonies may yield insight into the nutritional basis of caste. Epiponines and swarm-founding Ropalidiini are particularly important subjects for studies of the socio-nutritional basis of caste. Because these species do not go through a foundress period without workers, variation in the feeding rate of larvae over the course of the colony cycle should be less in these groups than in independent-founding species. Richards & Richards (92, p. 16) suggested that males and queens are produced simultaneously in epiponines after a period of worker production, but they did not present data to support this claim.

Nest architecture can provide cues for adults regarding the caste fate of female larvae. In Vespinae, cells used for rearing queens are larger than worker cells (37, 104), although queens are sometimes reared in expanded worker cells under favorable nutritional conditions (106). Cells of different size for rearing queens have also been recorded in the polistines *R. ignobilis* (125) and *Agelaia vicina* (145). Variation in cell size has been observed in nests of *Agelaia xanthopus* (a small percentage of cells scattered across combs were noticeably wide; S O'Donnell, personal observation) and *Agelaia yepocapa* (some pupal cell caps were taller; S O'Donnell & J Hunt, personal observations), but whether these larger cells were used to rear queens was not determined.

In part because of information on developmental pathways leading to worker caste divergence in ants (134), allometric growth associated with overall body size differences was assumed to be the evolutionary foundation of morphological castes. However, in epiponines, morphological caste differences are sometimes based on divergent body shapes rather than on overall size divergence. For this reason, Jeanne and colleagues (51, 53) have suggested that changes in the relative growth rates of imaginal discs (77) underlie the development of different castes and that this type of developmental reprogramming preceded differences in queen/worker body size in the group. If correct, this hypothesis raises interesting problems for the interpretation of the role of differential nutrition in bringing about caste differences, at least in the Epiponini. If queens and workers do not differ in size, then simple differences in the amount of food received by larvae may be inadequate to explain the production of castes. The role of differences in food quality, including the possibility of caste-determining oral secretions from adult wasps, has not been investigated. However, queens and workers that do not differ in overall length may

still differ in body mass. Measurements of the body mass of newly emerged queens and workers (before they are fed as adults) could be used to determine more accurately whether queens are in fact larger (or smaller) overall than workers.

Few studies have directly addressed nutritional allocation by vespid colonies. Roughly 40% of the total nitrogen arriving at *P. chinensis* colonies was invested in reproductives. This percentage appeared to decrease slightly with colony size, although the statistical significance of this decrease was not tested (111). Field experiments by Rossi & Hunt (95) showed that food supplementation (honey added to cells) to *P. metricus* nests led to elevated fat content in the first adults to emerge. Fat stores of wasps emerging from supplemented colonies were greater than those of their foundresses, suggesting that this aspect of the offsprings' physiology was typical of gynes rather than of workers (25). Hunt (43) further noted that adults emerging from *P. metricus*, *M. flavitarsus*, and *Mischocyttarus mexicanus* colonies in the laboratory were more likely to remain resident on their nests and act as workers when food was limited; in contrast, females often left colonies that were fed ad libitum within a few days of emergence and remained inactive away from the nest. Further studies suggest that *P. metricus* adults from supplemented colonies in the field behaved similarly (J Hunt, personal communication).

Experimental manipulation of food availability to laboratory *P. dominulus* colonies led to changes in colony development (68). The mean time to worker emergence was greater in food-limited colonies than in colonies supplied with prey ad libitum. However, the time to adult emergence of the first three workers did not differ among treatments. Destruction of brood, some of which were fed to nest-mate larvae, was greater in the food-limited colonies. Foundresses in food-deprived colonies apparently modified feeding patterns of larvae such that a small number of workers were produced rapidly. Among the adult offspring produced by single-foundress *P. chinensis antennalis* colonies before worker emergence, the first several to emerge had the shortest development times and often the smallest body sizes (74). These results suggest that *Polistes* spp. foundresses partition resources among their first offspring so as to produce a few small-bodied workers quickly.

Other Possible Factors in Pre-Imaginal Caste Determination

Both temperature and amount of food affect development time and adult body size in most ectotherms, including insects, and these factors can interact in complex ways (5, 8). Many species mature later at smaller body sizes when food quality or quantity is low, but later at larger body sizes when temperature is low. *Polistes foederatus* females from colonies fed ad libitum had longer larval development times after exposure to low temperatures (5°C) in the evening than

workers reared at more seasonal temperatures. Females emerging from cooled colonies were also larger and had better developed fat bodies, although they did not differ significantly in behavior from females in control colonies (120). Teasing apart the effects of temperature and larval nutrition may be important to studies of caste determination.

The vast majority of Vespidae known to exhibit morphological caste differences are housed in enclosed nests (Vespinae, swarm-founding *Ropalidia*, *Polybioides*, and Epiponini excluding *Apoica*; Table 1). The evolution of complex nest architecture in the ancestors of the Vespinae and most swarm-founding Polistinae may have allowed more precise social control over the caste of offspring females relative to species with open-comb nests. Enclosed nests are more homeostatic and may reduce the effects of temperature on variance in offspring size and developmental period. If the effects of temperature are diminished, then food supply likely becomes the single most important variable in affecting differential larval development.

Single gene effects on caste bias have been demonstrated in the socially parasitic ant *Harpagoxenus sublaevis* (137). Although environmental effects play a major role, genetic differences affecting caste determination have not been ruled out in Vespidae. Saito (96) argued that alleles reducing female fertility are the class of deleterious genes most likely to be maintained in haplodiploid populations where helping behavior is an option.

The possibility of maternal effects on caste via hormonal differences in recently laid eggs, as has been demonstrated in ants (114), has not been explored in Vespidae.

CONCLUSION: IMPLICATIONS FOR SOCIAL EVOLUTION

An important evolutionary implication of pre-imaginal caste determination is that helping behavior entails low fitness costs in workers with reduced fertility (128). To the extent that castes differentiate pre-imaginally, workers are not reproductively totipotent, and social options for some females are constrained from the outset of adult life. Much social conflict may be played out in the larval stage (79), and reduced plasticity in reproductive behavior for worker adults can mitigate conflicts of interest within societies. Immobile wasp larvae are likely at a great disadvantage relative to their attending adults (but are not necessarily helpless pawns: 42, 43). Manipulation of offspring into a helping role by mothers or other relatives is potentially great under these conditions (22, 128). Models have shown that the evolution of helping behavior is favored if its expression is facultative, as would be expected under conditions of female subfertility (20).

Ratneiks & Reeve (87) pointed out that workers can counter pre-imaginal manipulation of their fertility when they control production of reproductives nepotistically (by producing males themselves, biasing the queen's sex ratio, or by investing more in closely related reproductive females). However, actual conflict within societies can be prevented if it is costly. The extent to which worker subfertility is a result of manipulation, versus a response to prevailing ecological conditions, can be tested by manipulating food availability to colonies (43, 44, 68).

The studies summarized here demonstrate that reproductive caste differentiation often occurs before adult eclosion in eusocial Vespidae. Some females emerge as workers and are incapable of producing fertilized eggs; another set of females emerge as potential queens (gynes), with dominance contests acting on intracaste biases to demote some individuals from queen status. Interestingly, losers in social competition over reproductive status often disappear from colonies. This has been noted in epiponines (130, 131) and is also a frequent pattern in *Polistes* colonies (34, 85, but see 40). Even in species in which the capacity to reproduce is in part determined by interactions among adult females, entering the worker caste may not be an alternative open to many gynes that lose under social competition.

Some authors have suggested that unpredictably variable ecological and social conditions should select against morpho-anatomical specialization in behavior and social roles (40, 124). However, if reproductive caste is determined by divergent developmental pathways, then the relationship between structure and reproductive role may be canalized and therefore less responsive to selection for behavioral flexibility. The degree of morphological difference between queens and workers does appear to be evolutionarily labile. Solís & Strassmann (102) argue that unpredictable social and ecological environments favor caste flexibility over pre-imaginal determination. If variation in larval nutrition is the cause of caste differentiation, the worker caste may not only result from strategic manipulation of offspring by queens but rather may also represent an unavoidable developmental outcome in food-poor environments. The range of social options available to adult wasps within a population may depend partly on local nutritional ecology. If this is the case, then the timing of reproduction (emergence of gynes) during colony development should track changing social and environmental conditions, rather than being programmed by colony-level selection to occur at a particular stage or season (82, 113).

Although differential larval nutrition is implicated, the mechanisms of pre-imaginal determination are in need of further attention. Food supplementation experiments will prove valuable in addressing this issue. Supplementation experiments by Rossi & Hunt (95) are intriguing but difficult to interpret, given that the way supplemental food was handled and partitioned by adults at the

nest and the behavioral outcomes for supplemented females are unknown. As a final note of caution, many laboratory studies of wasp behavior, including those addressing caste determination, have used ad libitum feeding of adults (101, 102). Unlimited feeding can be expected to alter dramatically the reproductive biology of females emerging from subject colonies.

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Literature Cited

1. Akre RD, Reed HC. 1983. Evidence for a queen pheromone in *Vespula* (Hymenoptera: Vespidae). *Can. Entomol.* 115:371-77
2. Alexander RD. 1974. The evolution of social behavior. *Annu. Rev. Ecol. Syst.* 5:325-83
3. Andersson M. 1984. The evolution of eusociality. *Annu. Rev. Ecol. Syst.* 15:165-89
4. Archer ME. 1972. The significance of worker size in the seasonal development of the wasps *Vespula vulgaris* (L.) and *Vespula germanica* (F.). *J. Entomol. A* 46:175-83
5. Atkinson D. 1994. Temperature and organism size—a biological law for ectotherms? *Adv. Ecol. Res.* 25:1-58
6. Barbosa P. 1974. *Manual of Basic Techniques in Insect Histology*. Amherst: Autumn
7. Batra SWT. 1966. Nests and social behavior of halictine bees of India. *Indian J. Entomol.* 28:375-93
8. Berrigan D, Charnov EL. 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* 70:474-78
9. Blacklith RE. 1958. An analysis of polymorphism in social wasps. *Insectes Soc.* 5:263-72
10. Bohm MFK. 1972. Effects of environment and juvenile hormone on ovaries of the wasp *Polistes metricus*. *J. Insect Physiol.* 18:1875-83
11. Bourke AFG. 1988. Worker reproduction in the higher eusocial Hymenoptera. *Q. Rev. Biol.* 63:291-311
12. Brian MV. 1979. Caste differentiation and division of labor. In *Social Insects*, ed. HR Hermann, 1:121-222. New York: Academic
13. Carpenter JM. 1988. The phylogenetic system of the Stenogastrinae (Hymenoptera: Vespidae) *J. N. Y. Entomol. Soc.* 96:140-75
14. Carpenter JM. 1989. Testing scenarios: wasp social behavior. *Cladistics* 5:131-44
15. Carpenter JM. 1991. Phylogenetic relationships and the origin of social behavior in the Vespidae. See Ref. 94a, pp. 7-32
16. Carpenter JM. 1993. Biogeographic patterns in the Vespidae (Hymenoptera): two views of Africa and South America. In *Biological Relationships Between Africa and South America*, ed. P Goldblatt, pp. 139-55. New Haven: Yale Univ. Press
17. Carpenter JM, Ross KG. 1984. Colony composition in four species of Polistinae from Suriname, with a description of the larva of *Brachygastra scutellaris*

- (Hymenoptera: Vespidae). *Psyche* 91: 237–50
18. Chandrashekara K, Gadagkar R. 1991. Behavioural castes, dominance and division of labour in a primitively eusocial wasp. *Ethology* 87:269–83
 19. Cowan D. 1991. The solitary and presocial Vespidae. See Ref. 94a, pp. 33–73
 20. Craig R. 1983. Subfertility and the evolution of eusociality by kin selection. *J. Theor. Biol.* 100:379–97
 21. Crespi BJ, Yanega D. 1995. The definition of eusociality. *Behav. Ecol.* 6:109–15
 22. Dani FR. 1994. Caste size differences in *Polistes gallicus* (L.) (Hymenoptera: Vespidae). *Ethol. Ecol. Evol.* NS 3:67–73
 23. de Kort CAD. 1990. Juvenile hormone and insect reproduction. *Adv. Invert. Reprod.* 5:187–92
 24. de Wilde J, Beetsma J. 1982. The physiology of caste development in social insects. *Adv. Insect Physiol.* 16:167–246
 25. Eickwort K. 1969. Separation of the castes of *Polistes exclamans* and notes on its biology (Hym.: Vespidae). *Ins. Soc.* 16:67–72
 26. Emlen ST. 1991. Cooperative breeding in birds and mammals. In *Behavioural Ecology: An Evolutionary Approach*, ed. JR Krebs, NB Davies, pp. 301–37. Oxford: Blackwell. 2nd ed.
 27. Evans HE. 1958. The evolution of social life in wasps. In *Proc. 10th Int. Cong. Entomol.*, pp. 449–57
 28. Evans HE, West-Eberhard MJ. 1970. *The Wasps*. Ann Arbor: Univ. Mich. Press
 29. Fletcher DJC, Ross KG. 1985. Regulation of reproduction in eusocial Hymenoptera. *Annu. Rev. Entomol.* 30:19–43
 30. Francescato E, Baldini Tosi A, Turillazzi S. 1994. Caste dimorphism in *Polybioides tabidus* and *P. raphigastra*. In *Les Insectes Sociaux, Proc. 12th Int. Congr., Int. Union Study Soc. Insects*, ed. A Lenoir, G Arnold, M LePage, p. 424. Paris: Publ. Univ. Paris Nord
 31. Gadagkar R. 1994. Why the definition of eusociality is not helpful to understand its evolution and what we should do about it. *Oikos* 70:485–88
 32. Gadagkar R, Bhagavan S, Chandrashekara K, Vinutha C. 1991. The role of larval nutrition in pre-imaginal biasing of caste in the primitively eusocial wasp *Ropalidia marginata* (Hymenoptera: Vespidae). *Ecol. Entomol.* 16:435–40
 33. Gadagkar R, Vinutha C, Shanubhogue A, Gore AP. 1988. Pre-imaginal biasing of caste in a primitively eusocial insect. *Proc. R. Soc. Lond. B* 233:175–89
 34. Gamboa GJ, Heacock BD, Wiltjer SL. 1978. Division of labor and subordinate longevity in foundress associations of the paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 51:343–52
 35. Grechka EO. 1986. The regulation of seasonal development and caste determination in *Polistes* wasps. Transl. C Starr, LD Kassianoff. In *Essays in Memory of N.A. Kholodkoshii*, ed. VB Zinchuk, pp. 43–73. Leningrad: Nauka
 36. Grechka EO, Kipyatkov VY. 1983. Seasonal developmental cycle and caste differentiation in the social wasp *Polistes gallicus* L. (Hymenoptera, Vespidae). I. Phenology and life cycle regulation. *Entomol. Rev.* 62:19–31
 37. Greene A. 1991. *Dolichovespula* and *Vespula*. See Ref. 94a, pp. 263–305
 38. Haggard CM, Gamboa GJ. 1980. Seasonal variation in body size and reproductive condition of a paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). *Can. Entomol.* 112:239–48
 39. Hölldobler B, Wilson EO. 1990. *The Ants*. Cambridge: Harvard Univ. Press
 40. Hughes CR, Beck MO, Strassmann JE. 1987. Queen succession in the social wasp, *Polistes annularis*. *Ethology* 76:124–32
 41. Hughes CR, Strassmann JE. 1988. Age is more important than size in determining dominance among workers in the primitively eusocial wasp, *Polistes instabilis*. *Behaviour* 107:1–15
 42. Hunt JH. 1988. Lobe erection behavior and its possible social role in larvae of *Mischocyttarus* paper wasps. *J. Insect Behav.* 1:379–86
 43. Hunt JH. 1991. Nourishment and the evolution of the social Vespidae. See Ref. 94a, pp. 426–50
 44. Hunt JH. 1994. Nourishment and evolution in wasps *sensu lato*. In *Nourishment and Evolution in Insect Societies*, ed. JH Hunt, CA Nalepa, pp. 211–44. Boulder: Westview
 45. Hunt JH, Baker I, Baker HG. 1982. Similarity of amino acids in nectar and larval saliva: the nutritional basis for trophallaxis in social wasps. *Evolution* 36:1318–22
 46. Hunt JH, Schmidt DK, Mulkey SS, Williams MA. 1996. Caste dimorphism in the wasp *Epipona guerini* (Hymenoptera: Vespidae, Polistinae, Epiponini): further evidence for larval determination. *J. Kans. Entomol. Soc.* 69:362–69 (Suppl.)
 47. Itô Y. 1993. *Behaviour and Social Evolution of Wasps*. Oxford: Oxford Univ. Press

48. Jeanne RL. 1972. Social biology of the Neotropical wasp *Mischocyttarus drewseni*. *Bull. Mus. Comp. Zool. Harvard Univ.* 144:63–150
49. Jeanne RL. 1980. Evolution of social behavior in the Vespidae. *Annu. Rev. Entomol.* 25:371–96
50. Jeanne RL. 1991. Polyethism. See Ref. 94a, pp. 389–425
51. Jeanne RL. 1996. Non-allometric queen-worker dimorphism in *Pseudopolybia difficilis* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 69:370–74 (Suppl.)
52. Jeanne RL, Fagen R. 1974. Polymorphism in *Stelopolybia areata* (Hymenoptera, Vespidae). *Psyche* 81:155–66
53. Jeanne RL, Graf CA, Yandell BS. 1995. Non-size based morphological castes in a social insect. *Naturwissenschaften* 82:296–98
54. Keller L. 1995. Parasites, worker polymorphism, and queen number in social insects. *Am. Nat.* 145:842–47
55. Keller L, Perrin N. 1995. Quantifying the level of eusociality. *Proc. R. Soc. London B* 260:311–15
56. Kojima J. 1996. Colony cycle of an Australian swarm-founding paper wasp, *Ropalidia romandi* (Hymenoptera: Vespidae). *Ins. Soc.* 43:411–20
57. Kukuk P. 1994. Replacing the terms 'primitive' and 'advanced': new modifiers for the term 'eusocial'. *Anim. Behav.* 47:1475–78
58. Landolt PJ, Akre RD, Greene A. 1977. Effects of colony division on *Vespula atropilosa* (Sladen) (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 50:135–47
59. Lin NL, Michener CD. 1972. Evolution of sociality in insects. *Q. Rev. Biol.* 47:131–59
60. Litte M. 1977. Behavioral ecology of the social wasp, *Mischocyttarus mexicanus*. *Behav. Ecol. Sociobiol.* 2:229–46
61. Litte M. 1979. *Mischocyttarus flavitarsis* in Arizona: social and nesting biology of a polistine wasp. *Z. Tierpsychol.* 50:282–312
62. Litte M. 1981. Social biology of the polistine wasp *Mischocyttarus labiatus*: survival in a Colombian rain forest. *Smithson. Contrib. Zool.* 327:1–27
63. Lorenzi MC, Turillazzi S. 1986. Behavioral and ecological adaptations to the high mountain environment of *Polistes biglumus bimaculatus*. *Ecol. Entomol.* 11:199–204
64. Machado VLL, Gravena S, Giannotti E. 1988. Análise populacional e morfométrica em uma colônia de *Brachygastra lecheguana* (Latreille, 1824) na fase reprodutiva. *Ann. Soc. Entomol. Bras.* 17:491–506
65. Matsuura M, Yamane S. 1990. *Biology of the Vespine Wasps*. New York: Springer
66. Mead F, Gabouriaut D. 1993. Post-eclosion sensitivity to social context in *Polistes dominulus* Christ females (Hymenoptera, Vespidae). *Ins. Soc.* 40:11–20
67. Mead F, Gabouriaut D, Habersetzer C. 1995. Nest-founding behavior induced in the first descendants of *Polistes dominulus* Christ (Hymenoptera: Vespidae) colonies. *Ins. Soc.* 42:385–96
68. Mead F, Habersetzer C, Gabouriaut D, 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
69. Michener CD. 1969. Comparative social behavior of bees. *Annu. Rev. Entomol.* 14:299–342
70. Michener CD. 1974. *The Social Behavior of the Bees: A Comparative Study*. Cambridge: Harvard Univ. Press
71. Miyano S. 1980. Life tables of colonies and workers in a paper wasp, *Polistes chinensis antennalis*. *Res. Pop. Ecol.* 22:69–88
72. 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.* 25:198–209
73. Miyano S. 1986. Colony development, worker behavior and male production in orphan colonies of a Japanese paper wasp, *Polistes chinensis antennalis* Pérez (Hymenoptera: Vespidae). *Res. Popul. Ecol.* 28:347–361
74. Miyano S. 1990. Number, larval durations and body weights of queen-reared workers of a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera, Vespidae). *Nat. Hist. Res.* 1:93–97
75. Miyano S. 1991. Worker reproduction and related behavior in orphan colonies of a Japanese paper wasp, *Polistes jadvigae* (Hymenoptera: Vespidae). *J. Ethol.* 9:135–46
76. Mueller UG. 1996. Life history and social evolution of the primitively eusocial bee *Augochlorella striata* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 69:116–38 (Suppl.)
77. Nijhout HF, Wheeler DE. 1996. Growth models of complex allometries in holometabolous insects. *Am. Nat.* 148:40–56

78. Nonacs P, Reeve HK. 1995. The ecology of cooperation in wasps: causes and consequences of alternative reproductive decisions. *Ecology* 76:953–67
79. Nonacs P, Tobin JE. 1992. Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* 46:1605–20
80. O'Donnell S. 1996. Reproductive potential and division of labor in wasps: Are queen and worker behavior alternative strategies? *Ethol. Ecol. Evol.* 8:305–8
81. O'Donnell S. 1997. How parasites can promote the expression of social behaviour in their hosts. *Proc. Royal Soc. Lond. B* 264:689–94
82. Oster GF, Wilson EO. 1978. *Caste and Ecology in the Social Insects*. Princeton: Princeton Univ. Press
83. Pardi L. 1948. Dominance order in *Polistes* wasps. *Physiol. Zool.* 21:1–13
84. Pardi L, Marino Piccoli MT. 1981. Studies on the biology of *Belonogaster* (Hymenoptera Vespidae). 4. On caste differences and the position of this genus among social wasps. *Monit. Zool. Ital. NS* 9:131–46
- 84a. Peeters C, Crozier RH. 1988. Caste and reproduction in ants: Not all mated egg-layers are “queens.” *Psyche* 95:283–88
85. Pfennig DW, Klahn JE. 1985. Dominance as a predictor of cofoundress disappearance order in social wasps (*Polistes fuscatus*). *Z. Tierpsychol.* 67:198–203
86. Pratte M. 1993. Experimental changes of hierarchical rank in *Polistes dominulus* Christ foundresses. *Ethology* 95:97–104
87. Ratneiks FLW, Reeve HK. 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol.* 158:33–65
88. Reed HC, Gallego J, Nelson J. 1988. Morphological evidence for polygyny in post-emergence colonies of the red paper wasp, *Polistes perplexus* Cresson (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 61:453–63
89. Richards OW. 1969. The biology of some W. African social wasps (Hymenoptera: Vespidae, Polistinae). *Mem. Soc. Entomol. Ital.* 48:79–93
90. Richards OW. 1971. The biology of the social wasps (Hymenoptera, Vespidae). *Biol. Rev.* 46:483–528
91. Richards OW. 1978. *The Social Wasps of the Americas*. London: Br. Mus. Nat. Hist.
92. Richards OW, Richards MJ. 1951. Observations on the social wasps of South America (Hymenoptera Vespidae). *Trans. R. Entomol. Soc. Lond.* 102:1–169
93. Robinson GE. 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37:637–65
94. Ross KG. 1985. Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). *J. Zool. Lond. A* 205:411–24
- 94a. Ross KG, Matthews RW, eds. 1991. *The Social Biology of Wasps*. Ithaca: Cornell Univ. Press
95. Rossi AM, Hunt JH. 1988. Honey supplementation and its developmental consequences: evidence for food limitation in a paper wasp, *Polistes metricus*. *Ecol. Entomol.* 13:437–42
96. Saito Y. 1994. Is sterility by deleterious recessives an origin of inequalities in the evolution of eusociality? *J. Theor. Biol.* 166:113–15
97. Sakagami SF, Zucchi R, Yamane SO, Noll FB, Camargo JMP. 1996. Morphological caste differences in *Agelais vicina*, the Neotropical swarm-founding polistine wasp with the largest colony size among social wasps (Hymenoptera: Vespidae). *Sociobiology* 28:207–23
98. Schmid-Hempel P. 1995. Parasites and social insects. *Apidologie* 26:255–71
99. Sherman PW, Lacey EA, Reeve HK, Keller L. 1995. The eusociality continuum. *Behav. Ecol.* 6:102–8
100. Shima SN, Yamane S, Zucchi R. 1994. Morphological caste differences in some Neotropical swarm-founding polistine wasps. I. *Apoica flavissima* (Hymenoptera: Vespidae). *Jpn. J. Entomol.* 62:811–22
101. Sinha A, Premnath S, Chandrashekar K, Gadagkar R. 1993. *Ropalidia rufoplagiata*: a polistine wasp society probably lacking permanent reproductive division of labour. *Ins. Soc.* 40:69–86
102. Solís CR, Strassmann JE. 1990. Presence of brood affects caste differentiation in the social wasp, *Polistes exclamans* Viereck (Hymenoptera: Vespidae). *Funct. Ecol.* 4:531–41
103. Spradbery JP. 1972. A biometric study of seasonal variation in worker wasps (Hymenoptera: Vespidae). *J. Entomol. A* 47:61–69
104. Spradbery JP. 1973. *Wasps*. Seattle: Univ. Wash. Press
105. Spradbery JP. 1986. Polygyny in the Vespinae with special reference to the hornet *Vespa affinis picea* Buysson (Hymenoptera Vespidae) in New Guinea. *Monit. Zool. Ital. NS* 20:101–18

106. Spradbery JP. 1993. Queen brood reared in worker cells by the social wasp, *Vespula germanica* (F.) (Hymenoptera: Vespidae). *Ins. Soc.* 40:181-90
107. Strambi A. 1985. Physiological aspects of caste differentiation in social wasps. In *Caste Differentiation in Social Insects*, ed. JAL Watson, BM Okot-Kotber, C Noirot, pp. 371-84. Oxford: Pergamon
108. Strassmann JE, Lee RE, Rojas RR, Baust JG. 1984. Caste and sex differences in cold-hardiness in the social wasps *Polistes annularis* and *P. exclamans* (Hymenoptera: Vespidae). *Ins. Soc.* 31:291-301
109. Strassmann JE, Meyer DC. 1983. Gerontocracy in the social wasp, *Polistes exclamans*. *Anim. Behav.* 31:431-38
110. Strassmann JE, Queller DC, Solís CR, Hughes CR. 1991. Relatedness and queen number in the Neotropical wasp, *Parachartergus colobopterus*. *Anim. Behav.* 42:461-70
111. Suzuki T. 1981. Flesh intake and production of offspring in colonies of *Polistes chinensis antennalis* (Hymenoptera, Vespidae). *Kontyû* 49:283-301
112. Suzuki T. 1985. Mating and laying of female-producing eggs by orphaned workers of a paper wasp, *Polistes snelleni* (Hymenoptera: Vespidae). *Ann. Entomol. Soc. Am.* 78:736-39
113. Suzuki T. 1986. Production schedules of males and reproductive females, investment sex ratios, and worker-queen conflict in paper wasps. *Am. Nat.* 128:366-78
114. Suzzoni JP, Passera L, Strambi A. 1980. Ecdysteroid titre and caste determination in the ant *Pheidole pallidula* (Nyl.) (Hym., Form.). *Experientia* 36:1228-29
115. Trivers RL. 1985. *Social Evolution*. Menlo Park, CA: Benjamin/Cummings
116. Tsuji K. 1992. Sterility for life: applying the concept of eusociality. *Anim. Behav.* 44:572-73
117. Turillazzi S. 1980. Seasonal variations in the size and anatomy of *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Monit. Zool. Ital. NS* 14:63-75
118. Turillazzi S. 1989. The origin and evolution of social life in the Stenogastrinae (Hymenoptera, Vespidae). *J. Insect Behav.* 2:649-61
119. Turillazzi S. 1991. The Stenogastrinae. See Ref. 94a, pp. 74-98
120. Turillazzi S, Conte A. 1981. Temperature and caste differentiation in laboratory colonies of *Polistes foederatus* (Kohl) (Hymenoptera Vespidae) *Monit. Zool. Ital. NS* 15:275-97
121. Turillazzi S, Francescato E, Baldini Tosi A, Carpenter JM. 1994. A distinct caste difference in *Polybioides tabidus* (Fabricius) (Hymenoptera: Vespidae). *Ins. Soc.* 41:327-30
122. Turillazzi S, Pardi L. 1977. Body size and hierarchy in polygynic nests of *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Monit. Zool. Ital. NS* 11:101-12
123. Turillazzi S, Pardi L. 1982. Social behavior of *Parischnogaster nigricans serrei* (Hymenoptera: Vespoidea) in Java. *Ann. Entomol. Soc. Am.* 75:657-64
124. Villet MH. 1992. Definitions of "caste" in social insects. *Ethol. Ecol. Evol.* 4:213-24
125. Wenzel JW. 1992. Extreme queen-worker dimorphism in *Ropalidia ignobilis*, a small-colony wasp (Hymenoptera: Vespidae). *Insectes Soc.* 39:31-43
126. Wenzel JW, Carpenter JM. 1994. Comparing methods: adaptive traits and tests of adaptation. In *Phylogenetics in Ecology*, ed. P Eggleton, R Vane-Wright, pp.79-101. London: Harcourt Brace
127. West-Eberhard MJ. 1969. The social biology of polistine wasps. *Misc. Pub. Mus. Zool. Univ. Mich.* 140:1-101
128. West-Eberhard MJ. 1975. The evolution of social behavior by kin selection. *Q. Rev. Biol.* 50:1-33
129. West-Eberhard MJ. 1978. Polygyny and the evolution of social behavior in wasps. *J. Kans. Entomol. Soc.* 51:832-56
130. West-Eberhard MJ. 1978. Temporary queens in *Metapolybia* wasps: nonreproductive helpers without altruism? *Science* 200:441-43
131. West-Eberhard MJ. 1981. Intragroup selection and the evolution of insect societies. In *Natural Selection and Social Behavior: Recent Research and New Theories*, ed. RD Alexander, DW Tinkle, pp. 3-17. New York: Chiron
132. West-Eberhard MJ. 1987. Flexible strategy and social evolution. In *Animal Societies: Theories and Facts*, ed. Y Itô, JL Brown, J Kikkawa, pp. 35-51. Tokyo: Jpn. Sci. Soc.
133. Wheeler DE. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* 128:13-34
134. Wheeler DE. 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat.* 138:1218-38
135. Wilson EO. 1971. *The Insect Societies*. Cambridge: Harvard Univ. Press
136. Winston ML. 1987. *The Biology of the Honey Bee*. Cambridge: Harvard Univ. Press
137. Winter U, Buschinger A. 1986. Ge-

- netically mediated queen polymorphism and caste determination in the slave-making ant, *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). *Entomol. Gen.* 11:125-37
138. Yamane S. 1985. Social relations among females in pre- and postemergence colonies of a subtropical paper wasp, *Parapolybia varia* (Hymenoptera: Vespidae). *J. Ethol.* 3:27-38
139. Yamane S. 1986. The colony cycle of the Sumatran paper wasp *Ropalidia (Icariola) variegata jacobsoni* (Buysson), with reference to the possible occurrence of serial polygyny (Hymenoptera Vespidae). *Monit. Zool. Ital. NS* 20:135-61
140. Yamane S, Kojima J, Yamane SK. 1983. Queen/worker size dimorphism in an oriental polistine wasp, *Ropalidia montana* Carl (Hymenoptera: Vespidae). *Ins. Soc.* 30:416-22
141. Yamane S, Okazawa T. 1977. Some biological observations on a paper wasp, *Polistes (Megapolistes) tepidus malayanus* Cameron (Hymenoptera, Vespidae) in New Guinea. *Kontyû* 45:283-99
142. Yanega D. 1992. Does mating determine caste in sweat bees? (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 65:231-37
143. Yoshikawa K, Ohgushi R, Sakagami S. 1969. Preliminary report on entomology of the Osaka City University 5th scientific expedition to Southeast Asia 1966. *Nat. Life. Southeast Asia* 6:153-82
144. Zikán JF. 1951. Polymorphismus und Ethologie der sozialzen Faltenwespen (Vespidae Diptoptera). *Act. Zool. Lill.* 11:5-51
145. Zucchi R, Sakagami SF, Noll FB, Mechi MR, Mateus S, et al. 1995. *Agelaia vicina*, a swarm-founding polistine with the largest colony size among wasps and bees (Hymenoptera: Vespidae). *J. NY Entomol. Soc.* 103:129-37