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Evolution of castes in *Polistes*

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A novel hypothesis for the origin of castes in *Polistes* has recently been proposed: the worker and gyne castes among offspring of a *Polistes* colony are based on the underlying ground plan of reproductive physiology that would have characterized the non-diapause and diapause generations of a bivoltine solitary vespid wasp. Here the hypothesis is reviewed in light of the dynamic nature of diapause expression in insects in general, and it is re-named the diapause ground plan hypothesis. The diapause ground plan hypothesis differs in several significant aspects from an existing hypothesis for the origin of castes — the ovarian ground plan hypothesis. Phylogenetic implications of the diapause ground plan hypothesis also are in discord with the currently accepted classification of Vespidae, and the diapause ground plan hypothesis has been challenged for its failure to address multiple levels of analysis. Each of these areas of discord is described, and key points of difference between the diapause ground plan hypothesis and competing hypotheses are identified. Strong inference — the explicit testing of a hypothesis for possible rejection — is proposed as the most efficacious route to clearer understanding of the evolution of the castes in *Polistes*. Specific suggestions for some strong tests of competing hypotheses are proposed.

Introduction

The origin of a worker caste is the defining moment in the evolution of insect sociality (Fletcher & Ross 1985: p. 319). Given the long-recognized centrality of the paper wasp genus *Polistes* to an understanding of insect social evolution (Evans 1958), surprisingly little attention has been given to the origin of castes in that genus. It has long been known that the morphologically and behaviorally discrete worker and gyne castes of the hornet genus *Vespa* are determined during larval development (Ishay 1975), and similar ontogenetic caste determination probably occurs in all non-inquiline Vespinae (Matsuura & Yamane 1990). Morphologically and behaviorally discrete worker and gyne castes also characterize many swarm-founding Polistinae (Jeanne & Fagen 1974, Yamane *et al*. 1983, Turillazzi *et al*. 1994, Jeanne *et al*. 1995; for a review *see* Noll *et al*. 2004), and such differences can only arise during larval ontogeny. Ontogenetic dimorphism occurs even in some independent-founding polistines: *Ropalidia galimatia* (Wenzel 1992), *Belonogaster* (Pardi & Piccioli 1981, Keeping 2002), and, apparently, *Polistes olivaceous* (Alam 1958, Kundu 1967, cf. Miyano 1994). Given the near-universality of differentiation of even monomorphic Polistinae into worker and gyne behavioral castes

(Gadagkar 1991, Hunt 1991, 1994), and given the direct and consistent link between reproductive behavior and morphology in Vespinae and dimorphic swarm-founding Polistinae (Greene 1991, Jeanne 1991, Matsuura 1991), the notion of a single basis underlying and uniting behavioral and morphological castes of all members of both subfamilies is not unreasonable. Because *Polistes* is the sister taxon to all other Polistinae (Carpenter 1991), traits of its life history and social organization seem likely to closely reflect the ancestral state of the common ancestor of Polistinae + Vespinae; therefore *Polistes* would seem to be precisely where one should look for the foundations of caste difference, including mechanisms of caste determination. One is left to wonder, then, why not more attention has been focused on *Polistes* in pursuit of a subject of such fundamental importance.

Several reasons may underlie the paucity of attention to mechanisms of caste determination in *Polistes*. Foremost among these is the notion of caste totipotency in *Polistes* and other independent-founding polistines. If all adult females can become either a worker or queen depending on social context, then it can be argued that there would be no reason to expect, much less look for, an ontogenetic basis for caste determination. Widespread acceptance of the notion of caste totipotency may reflect in part the hegemony of relatedness-centered ways of understanding social evolution, because caste totipotency is the centerpiece, indeed the very foundation of that approach: "Consider a species where the female consecutively provisions and oviposits in cell after cell so that she is still at work when the first of her female offspring ecloses, leaves the nest and mates. Our principle [inclusive fitness + haplodiploidy] tells us that even if this new adult had a nest already constructed and vacant for her use she would prefer, other things being equal, returning to her mother's and provisioning a cell for the rearing of an extra sister to provisioning a cell for a daughter of her own" (Hamilton 1964: pp. 28–29). Caste totipotency does, in fact, characterize facultative sociality (Crespi & Yanega 1995) in hover wasps, the Stenogastrinae (Turillazzi 1991, Field *et al*. 2000). Because Stenogastrinae is hypothesized to be the sister taxon to Polistinae + Vespinae (Carpenter 1982, 1991, 2003; cf. Schmitz & Moritz 1998), a different line of argument for caste totipotency in *Polistes* would be that caste totipotency can be logically inferred to have characterized the social common ancestor of the three social subfamilies of Vespidae (if, indeed, they had a common ancestor [Hunt 2007]; *see* below). For one or both of these reasons, then, caste totipotency is widely believed to characterize *Polistes*.

A second reason for scant attention to mechanisms of caste determination in *Polistes* is confusion (and sometimes conflation) of two independent phenomena — caste determination among offspring and caste differentiation among adult nestmates, particularly among co-foundresses. The nourishment-caste link during larval development is widely known: "nutritional effects on reproductive caste have been documented in numerous taxa and appear to be widespread, if not universal, among eusocial insects. Differences in the amount of food provided to larvae may underlie many of the differences between reproductives and workers, especially in species exhibiting the common pattern of larger body size for reproductives" (O'Donnell 2003: p. 152). At the same time, however, "dominance interactions among paper wasp (*Polistes*) females, which often cooperate to start new colonies, determine which female acts as the sole reproductive. Subordinate *Polistes* females function as workers" (O'Donnell 2003: p. 152). A call for distinction between the two kinds of *Polistes* workers, called ontogenetic and subordinate by Hunt (1991), seemingly went unheeded, although different research paradigms often focus on one kind of worker or the other. The nutritional scenario put forward in Hunt (1991) has ontogenetic caste determination among offspring as its explicit focus, but the chapter entitled "*Polistes*" in the same volume tacitly has caste differentiation among adult co-foundresses as its main theme (Reeve 1991). The likelihood of a mechanistic basis for caste dichotomy among offspring thus has been obscured by the absence of an ontogenetic basis for caste differentiation among co-foundresses and the confusion/conflation of two separate caste phenomena.

Perhaps a third reason for scant attention to possible mechanisms of caste determination in *Polistes* could be a shortage of conceptual frameworks within which to pursue ontogenetic mechanisms. A candidate framework does exist — the ovarian ground plan hypothesis put forward by West-Eberhard (1987a, 1987b, 1988, 1992, 1996). In this hypothesis, nest-sharing adult females are the context for caste origins, and castes are proposed to be based on an underlying ovarian cycle of an ancestral adult solitary wasp. In this hypothesis, the ancestral adult solitary wasp was characterized by a cycle in which ovarian activation leading to oviposition is followed by a phase of ovarian quiescence during provisioning of the nest cell in which the wasp has oviposited, followed by reactivation of the ovary leading to the next oviposition event. West-Eberhard envisions an uncoupling of phases in this ovarian cycle among several nest-sharing adult females, with some individuals (ultimately, the queens) coming to specialize in the active ovary, reproductive phase and others (ultimately, the workers) coming to specialize in the inactive ovary, foraging phase. Subsequent evolution of a switch-like control system is hypothesized to then have occurred (West-Eberhard 1989). West-Eberhard's model, therefore, is based on a concept of caste divergence among adults rather than caste determination during larval ontogeny as the fundamental framework for caste evolution.

Another conceptual framework now exists. Hunt and Amdam (2005) hypothesized a specific mechanism that can link larval ontogeny and caste determination in *Polistes*. The proposed mechanism is the bifurcation of developmental pathways based on an underlying ground plan of dichotomy between non-diapausing and diapausing individuals of a bivoltine solitary ancestor. Differences in larval nourishment are proposed to be central to the switch-like divergence of the two developmental pathways, and the proposed underlying mechanism consists of pre-existing pleiotropic gene networks whose regulatory frameworks have been co-opted by cues stemming from the social environment rather than cues from the ambient environment. The strength of our confidence in the hypothesis is measured by our assertion that this hypothesis "illustrates, by specific example, that social evolution in insects can be fully — and finally — understood" (Hunt & Amdam 2005: p. 267). Saying so doesn't make it so, however. The considerable challenge of testing the hypothesis lies ahead, and some important differences between the hypothesis and current thought have not been addressed. My goals in the present contribution, therefore, are (1) to briefly review the new hypothesis and add a refinement, (2) to call attention to some of the major differences between the new hypothesis and current thought, and (3) to propose that explicit challenging of hypotheses with tests of rejection is the most rapid and most effective route to enhanced understanding. The three topics in discord that I will cover are the differences between the hypothesis and the ovarian ground plan hypothesis, differences between the hypothesis and the six-subfamily cladogram of relationships in Vespidae, and an address to a challenge that Amdam and I committed a levels-of-analysis error by putting forward only a mechanistic hypothesis. Before turning to those topics, I will briefly encapsulate and refine the hypothesis for the ontogenetic basis of worker and gyne castes in *Polistes*.

The diapause ground plan hypothesis

A novel hypothesis for the origin of worker and gyne castes in *Polistes* was introduced by Hunt and Amdam (2005) and is addressed in greater detail by Hunt (2007), from which the following synopsis is drawn. References in support of points not referenced here can be found in Hunt (2007).

The life cycle of *Polistes* (Fig. 1B) has the same structure as that of a partially bivoltine solitary wasp living in a seasonal environment (Fig. 1A). A *Polistes* queen's early-emerging female offspring have the physiology of solitary wasps that engage in reproduction soon after emergence, and later-emerging *Polistes* female offspring have the physiology of solitary wasps that do not enter into reproduction but instead enter quiescence and reproduce the following year (Fig. 2). Direct reproduction by early-emerging females that engage in alloparental care — the workers — has been deleted from the life cycle (Fig. 1). However, to recognize that the early-emerging females possess the physiology of wasps primed to reproduce provides a power-

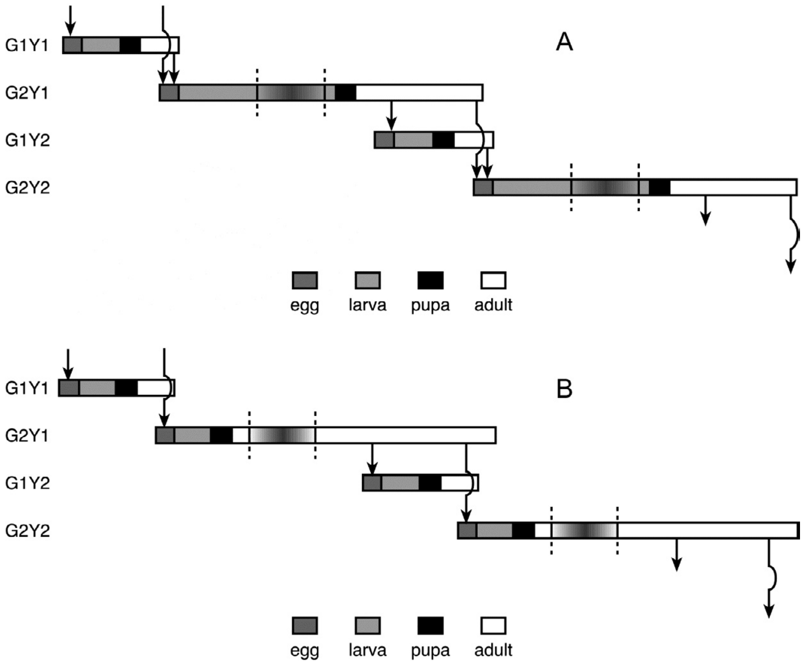

Fig. 1. — **A**: Life cycle of a partially bivoltine solitary wasp in a seasonal environment. Eggs of the first, non-diapause brood, G1, are laid by adults from the second generation of the preceding favorable season, G2. During the season that they begin as eggs, non-diapause females complete development, emerge as adults, and produce the diapause, G2, generation. In partial bivoltine life cycles, at least some diapause individuals also are offspring of the same females that lay eggs of the non-diapause offspring that preceded them (Seger 1983). All diapause G2 individuals then pass the unfavorable season, indicated by shading between the dotted vertical lines, in prepupal diapause, complete development at the onset of the following favorable season, emerge, mate, and initiate the next generation (O'Neill 2001). — **B**: Life cycle of *Polistes* in a seasonal environment. Basic life cycle components are the same as in partially bivoltine solitary wasps but with two major changes. One change is that diapause is passed as an adult rather than as a prepupa, which is known to occur in some bivoltine solitary wasps in family Sphecidae (references in O'Neill 2001). A second change is that offspring of the first generation typically do not reproduce but instead undertake allomaternal brood care at their natal nest, thus no arrow connects the non-diapause G1 and diapause G2 broods.

ful framework for understanding the origin and nature of worker behavior among the female offspring of a *Polistes* colony.

Worker behavior in *Polistes* is allomaternal behavior. Early-emerging *Polistes* female offspring are physiologically primed to reproduce, so they readily engage in maternal care, but the context of the colony into which they emerge as young adults shapes the maternal behavior that they express. Newly emerged *Polistes* cannot fly (Rau & Rau 1918: p. 256), or at least they do not leave the natal nest, or do so only briefly, for a period of up to several days after their emergence (Dew & Michener 1981, Strassmann *et al.* 1984, Post *et al.* 1988). By the time of their first foraging flight they will have fed upon larval saliva. (Jeanne [1972: p. 85] describes this particularly well for *Mischocyttarus*.) Because early-emerging female offspring are physiologically predisposed to seek protein for ovarian development, the amino-acid rich saliva (Hunt *et al.* 1982) of larvae in their natal nest becomes an attraction that induces them to remain. Roubaud's (1916) suggestion that attractiveness of larval saliva is what retains adults at the nest has been borne out by experimental studies (Ishay & Ikan 1968, Kumano & Kasuya 2001, Hunt & Dove 2002), and this attraction now appears to be deeply fundamental to the initiation of sociality. When early-emerged females undertake maternal behaviors, they do so at the place where they feed on larval saliva rather than at a nest

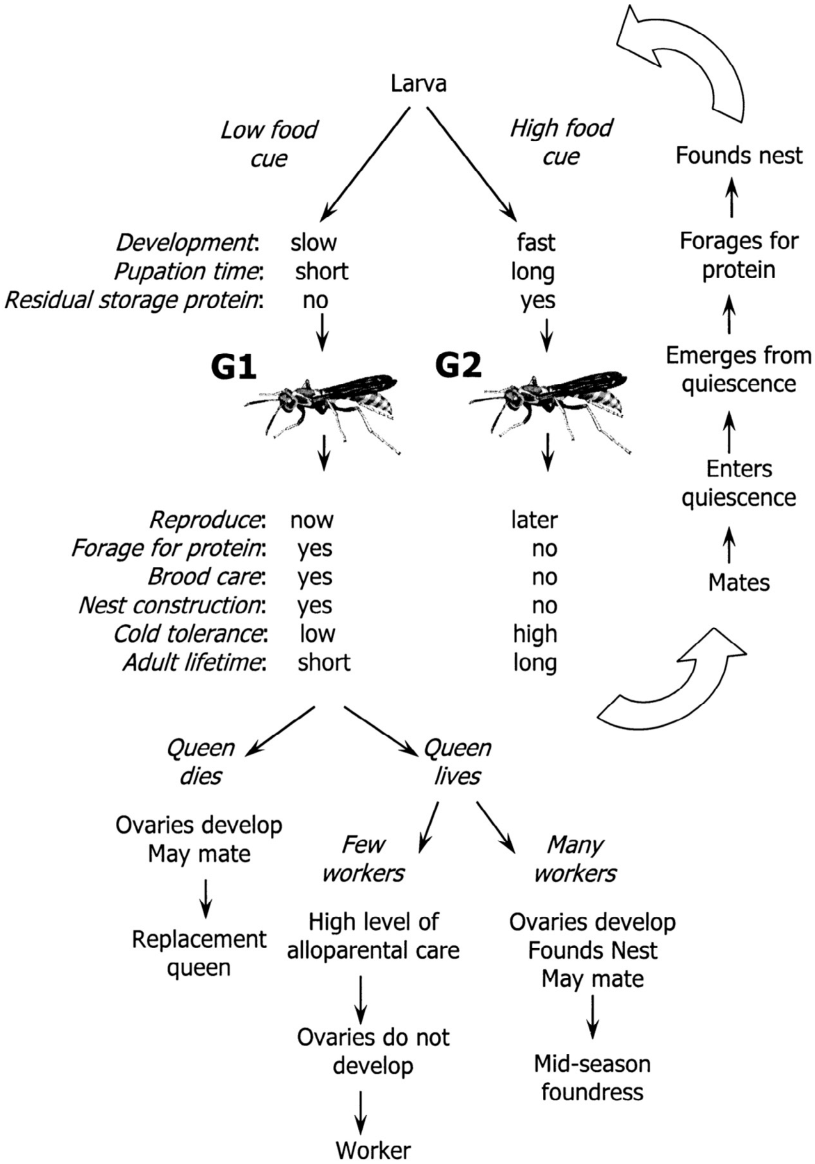

Fig. 2. The *Polistes* life cycle incorporates fundamental elements of the diapause ground plan. Larvae respond during development to a food cue and diverge onto one of two trajectories. Scanty provisioning leads to the non-diapause G1 pathway, which is signaled by slow larval development (due to low nutrient inflow), short pupation time (Karsai & Hunt 2002), and no storage protein residuum in emerging adults (Hunt *et al*. 2003). More abundant provisioning leads to more rapid larval development, longer pupation time (West Eberhard 1969, Karsai & Hunt 2002), and residual storage protein in diapause G2 adults (Hunt *et al*. 2003). Non-diapause G1 females have a "reproduce now" phenotype, and they forage for protein, care for brood, and construct nests. The expression of these behaviors is conditional, as indicated by branching points in the non-diapause G1 sequence. If the queen is lost, a non-diapause female can develop her ovaries, mate if males are present, and become a replacement queen. If a queen is present but the number of workers is low, a non-diapause female will alloparentally express maternal behaviors (i.e., nest construction, nest defense, brood care, and foraging) as a worker at her natal nest. Finally, if a queen is present and the number of workers is high, a non-diapause female may depart the natal nest and found a satellite nest in mid-season. Because cold tolerance of non-diapause females is low, they do not survive quiescence and lifetimes are short. In contrast, diapause G2 females have a "reproduce later" phenotype. They express no maternal behaviors the first year, but after emerging from quiescence they break reproductive diapause and shift to the "reproduce-now" phenotype. (Figure and legend from Hunt & Amdam 2005).

of their own making. This marks the threshold of sociality: they remain at their natal nest, and there they perform allomaternal care. This is worker behavior. Behavioral dominance of the queen and energetic costs of foraging and nest construction constrain or prevent egg production by these females, even though they emerged from pupation physiologically disposed to enter into reproduction.

The physiological disposition to reproduce in early-emerging females is borne out by the exceptions to worker behavior. Some early-emerging female *Polistes* may become replacement queens on their natal nest (Strassmann & Meyer 1983, Miyano 1986; Fig. 2), and others found satellite nests and lay eggs in them (Strassmann 1981, Page *et al.* 1989; Fig. 2) or become foundresses of replacement nests following nest destruction (Dani & Cervo 1992). Many of the wasps that undertake reproduction in these ways have previously been foragers (O'Donnell 1996).

When some females undertake allomaternal care, the female offspring that they care for are better nourished than were the females that were provisioned by their mother alone, and these better-nourished females emerge from pupation in reproductive diapause. These females are not disposed to enter into reproduction, and so they do not engage in maternal behaviors either directly or allomaternally. Although they will feed on larval saliva and on provisions brought by foragers to feed larval nestmates, the nourishment that these adult females receive is directed into storage rather than ovarian development. These females will mate with the males that emerge from pupation at about the same time they do, and they will pass the ensuing unfavorable season in quiescence. These females are gynes. When gynes emerge from quiescence at the start of the ensuing favorable (spring or rainy) season, they can become foundresses. If a foundress produces offspring, she becomes a queen.

The existence and basic nature of the two categories of *Polistes* female offspring are widely recognized, and they have been described by many students of *Polistes*, including Pardi (1948), Deleurance (1949, 1952), West-Eberhard (1969), and Miyano (1983). The two types of female offspring are sometimes thought of as "sterile" workers and "reproductive" gynes. This traditional terminology obscures the true nature of caste in social vespid wasps. The reproductive ability of early-emerging *Polistes* offspring and the absence of reproductive behaviors in later-emerging females have been documented in empirical studies by Bohm (1972) and Mead *et al.* (1995). In terms of reproductive physiology and its consequent behaviors during the season in which the *Polistes* offspring develop into adults, workers are reproductive and gynes are not. The gynes are in reproductive diapause.

Diapause is not a simple slowing down of metabolism; it is a specific developmental pathway different from non-diapause (Denlinger 2002). Gene expression differences specifically associated with diapause/non-diapause now have been reported in a diversity of insects, including beetles (de Kort & Koopmanschap 1994, Yocum 2001, 2003), moths (Yamashita 1996), and flies (Flannagan *et al.* 1998, Chen *et al.* 2005). In a number of cases a hexameric storage protein, as found in *Polistes* gynes (Hunt *et al.* 2003), has been specifically associated with the diapause phenotype (Šula *et al.* 1995, Kłudkiewicz *et al.* 1996). In the boll weevil, for example, diapause occurs in the adult stage, diapausing adults are characterized by expression of hexamerin, and the difference in non-diapause and diapause expression can be induced by differences in feeding of newly eclosed adults (Lewis *et al.* 2002). It is additionally important to note that there are taxa that exhibit intra-specific variation in diapause expression. For example, populations of a solitary wasp, *Trypoxylon politum* in the family Sphecidae, are partially bivoltine in the southeastern U.S., whereas populations north of central Virginia are univoltine (all diapause) (Brockmann 2004). Variation among partially bivoltine *T. politum* populations suggest a latitudinal gradient of responsiveness to environmental cues affecting diapause, and transfer of individuals between populations reveals plasticity of development as well as traces of local adaptation (Brockmann 2004).

If diapause is a ground plan component of many insects, and if diapause can be differentially expressed intra-specifically in response to environmental context — and both of these propositions are supported by living examples, then it is possible that diapause expression first appeared in the context of the proto-*Polistes* life history without the direct ancestor itself expressing diapause. That is, the ancestor of the proto-*Polistes* could have been multivoltine in a tropical environment. Given the architecture of the *Polistes* nest, with simultaneously open cells containing progressively provisioned larvae, the stage could have been set for emerged offspring to remain at their natal nest as a site where they could obtain proteinaceous nourishment for their own reproduction, much as seems to be the case in living Stenogastrinae (Hunt 2007). With the diapause pathway present in the ground plan, the stage would then have been set for a possible life history transition: if nourishment provisioned by alloparental first-emerged wasps to later-developing larvae was sufficient to trigger the diapause pathway in later-developing larvae, and if the wasps had been nesting in a seasonal environment in which producing a pool of gynes in diapause could have been demographically advantageous (Hunt 2007), the *Polistes* life cycle could have been established *de novo*. The novel invention would have been activation of the diapause pathway during larval development, leading to the gyne phenotype. Thus the immediate ancestor of *Polistes* need not have been bivoltine as was originally proposed by Hunt and Amdam (2005). It therefore is appropriate to characterize the hypothesis for the ground plan that underlies caste differentiation in *Polistes* as the diapause ground plan hypothesis rather than the bivoltine ground plan hypothesis.

Gynes become foundresses in the next favorable season. Haplometrosis (solitary founding) is the ancestral condition (Hunt 2007), and the ontogenetic partitioning of offspring females into ones that become workers at their natal nest and others that become gynes is the hallmark of sociality in Vespidae (Hunt 1991). For some species, nest founding involves pleometrosis (co-founding), wherein dominance interactions sort co-foundresses into dominant queens and subordinate workers (Pardi 1948). This behavioral partitioning among adult wasps that all had been gynes in the preceding season is not the same as the ontogenetic partitioning of offspring into workers and gynes, and it is based on different physiological phenomena (Hunt 2007).

The ovarian ground plan hypothesis

A model for the origin of social vespid wasps has long been proposed by West-Eberhard (1987a, 1987b, 1988, 1992, 1996). She hypothesizes (West-Eberhard 1996: p. 293): "the origin of reproductive 'castes' (queens and workers) in wasps might be classified as a 'reciprocal deletion,' for it probably involved the decoupling of ancestral reproductive cycle into two parts with one expressed in workers and the other in queens (West-Eberhard 1987a). Each caste lacks the 'deleted' set of traits expressed in the other, and the two alternatives are mutually 'dependent' or complementary morphs (*see* West-Eberhard 1979 and Gadagkar [1996]): they stay together and co-operate (or parasitize each other!) in the same colony, compensating each other's deficiencies." This scenario explicitly incorporates nest sharing by adults as the context: "the social wasps (Vespidae) probably arose from nest-building solitary ancestors, *via* a primitively social stage like that seen in many extant species, in which there is nest sharing by more than one conspecific female but no differentiation of associated females into egg-laying 'queens' and non-egg-laying 'workers' dedicated to brood care" (West-Eberhard 1987a: p. 38).

A solitary wasp exhibits cyclical reproductive behaviors: nest cell construction, ovipositioning, foraging and provisioning, then construction again. West-Eberhard envisions that if such a wasp has an underlying physiology such that it matures only one egg at a time in its ovary, then the components of the behavior cycle and its underlying physiology could be expressed differentially among nest-sharing adults. Some individuals in a nest-sharing group could come to express the ovipositional role and others could come to express the foraging and construction roles, leading to reproductive and worker castes (Fig. 3). As a potential mechanism, West-Eberhard (1996: p. 301) proposes that "[…] *a decoupling of the ovarian and behavioural influences of JH (juvenile hormone) in workers and queens could initially have been achieved by incidental and/or socially imposed nutritional differences between the two castes that differentiated their responses to JH, producing two classes of females* […]".

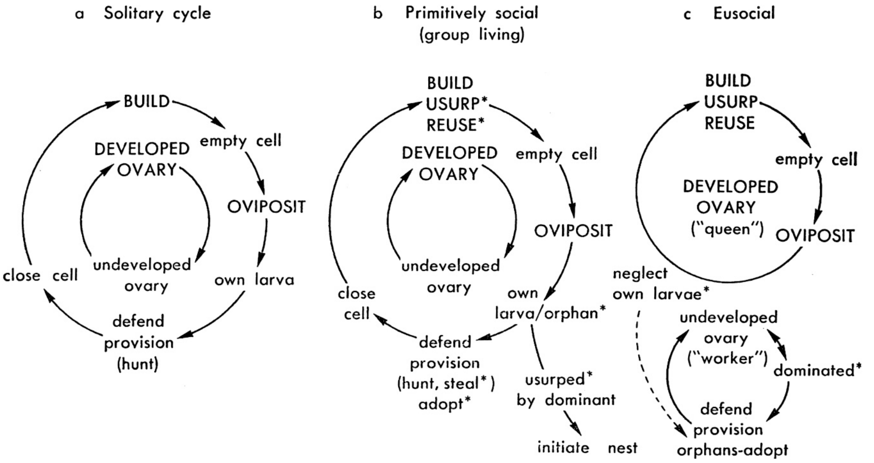

Fig. 3. Social evolution *via* contextual shift in wasps. **a** represents the ovarian and behavioural cycles of a progressively provisioning solitary wasp. **b** is based on observations of *Z[ethus] miniatus*, and could be derived from **a** *via* contextual change with the advent of group life under selection for remaining at the maternal nest. **c** represents a cycle like that of *Polistes* and other eusocial wasps in which selection for success in social competition has led to effective mechanisms of control of the reproduction of subordinate group members, with a consequent rise in the number of orphans as well as ovary-suppressed females inclined to adopt them. Asterisks (*) indicate novel occurrences that could be indirect or emergent results of selection producing **b** group living, and **c** effective dominance. (Figure and legend from West-Eberhard 1987a.)

There are differences between the ovarian ground plan and diapause ground plan hypotheses. First, in the ovarian ground plan hypothesis the worker caste is envisioned as evolving among nest-sharing females of a single generation, whereas in the diapause ground plan hypothesis the context for worker evolution is solitary nesting with mother-daughter interactions. To resurrect some terminology from the 1970s, the ovarian ground plan hypothesis is a semisocial (within-generation) model, and the diapause ground plan hypothesis is a subsocial (between-generation) model. Second, in the ovarian ground plan hypothesis the underlying physiology is proposed to be a continuous ovarian cycle in which reproductives come to reflect the ovary-activated, egg laying phase of the cycle, and workers come to reflect the ovary-inactive, foraging phase of the cycle. In the diapause ground plan hypothesis, the underlying physiology of caste difference is proposed to be the bifurcation of ontogenetic pathways expressed as non-diapause and diapause in solitary insects of many taxa in seasonal environments. Thus the ovarian ground plan hypothesis proposes that a switch-like mechanism evolved *de novo* simultaneously with or following caste divergence to serve that specific adaptive role, whereas the diapause ground plan hypothesis proposes that caste divergence is based on co-option of a pre-existing switch-like physiological mechanism.

Perhaps the fundamental difference between the diapause and ovarian ground plan hypotheses is that the ovarian ground plan hypothesis includes no concept of a gyne. This can be seen in the work of Giray *et al.* (2005), who report studies of *Polistes canadensis* that were framed by the ovarian ground plan hypothesis. Data presented in the text and in two figures reveal confusion of two commonly used terms. In the text, a set of studied reproductives are “queens from postemergence nests […]; queens of preemergence single foundress nests […]; and queens of multiple foundress nests” (p. 3333). In their fig. 4 (p. 3333), all of these are lumped together as “gynes.” The term “gyne” is not defined or used in their text, and in their fig. 5 (p. 3333) “gyne” is used interchangeably with “queen.”

Queens of social Hymenoptera are egg-laying, usually inseminated, behaviorally dominant, and usually non-foraging females in full reproductive mode. Throughout the literature on social Hymenoptera, “gyne” is a term has been used to characterize females that are worker-fed as larvae, that often differ in morphology from workers, and that are not reproductively active yet are specifically recognized as potential queens of the next generation (e.g., Tsuchida *et al*. 2003, Tarpy & Gilley 2004, Alaux *et al*. 2005, Chinh & Sommeijer 2005, Richards *et al*. 2005, Schlick-Steiner *et al*. 2005). In social wasps, gynes do not work or reproduce in the season of their development. Instead, they pass an unfavorable season in quiescence and then become working foundresses — and queens if they are fortunate — in the next favorable season. The use of the term “gyne” by Giray *et al*. (2005) does not fit or incorporate this concept. Giray *et al*. did, however, note that “*a possible interpretation of* […] *the failure to forage of numerous females by age 12 days is that a portion of newly emerging females in this population of P. canadensis during the periods (late wet season) of our observations and experiments were in reproductive diapause*” (p. 3334). It is probable that they were, and if so they were gynes as the term is used here and in the social Hymenoptera literature generally.

The ovarian and diapause ground plan hypotheses have a common thread — that nutritional differences can be the basis for phenotypic differentiation. The diapause ground plan hypothesis presents a plausible model for the origin of a switch mechanism that differentiates diapause (G1) and non-diapause (G2) pathways in larvae, but the original presentation of the idea (Hunt & Amdam 2005) did not address the physiology that would underlie the undertaking of maternal behaviors (alloparentally) by these wasps. Parts of the ovarian ground plan hypothesis more closely approach that component of the scenario. Both hypotheses have a common focus of investigation, and a synthesis of selected components of the hypotheses will lead to a robust scenario for both expressions of caste in *Polistes* — larval determination of two ontogenetic pathways and adult differentiation within each of those pathways. Adult differentiation occurs among non-diapause (G1) females as in Fig. 2 and among diapause (G2) females if they are co-foundresses.

The six subfamily hypothesis

The journal that published the Hunt and Amdam (2005) hypothesis received two letters to the editor that were critical of the hypothesis. Although these letters and our responses to them were not published, the points raised by the letter writers are ones that will have occurred to others, and so they merit discussion. One criticism is that the diapause ground plan hypothesis is at odds with the six-subfamily hypothesis of relationships among living Vespidae (Carpenter 1982, 1989, 1991, 2003). This criticism can be broken into two parts, bivoltine ancestry and eumenine ancestry.

It is true that there currently is no evidence to support the proposition that the ancestor of *Polistes* was bivoltine. The revision to the original Hunt and Amdam hypothesis that is given earlier in this paper and in Hunt (2007) renders this criticism moot. The revision was not made to quell criticism; it was made because the revised scenario of context-dependent activation of a diapause pathway latent in the ground plan is a more conservative hypothesis of the possible course of events. The revision does not rule out the possibility that the ancestor of *Polistes* was, in fact, bivoltine, but it does rule out voltinism of the ancestor as a test of the diapause ground plan hypothesis.

Eumenine ancestry of *Polistes* as proposed by Hunt and Amdam (2005) raises a more fundamental problem. In the six subfamily cladogram of relationships in Vespidae (Carpenter 1982, 1989, 1991, 2003; Fig. 4), the fully social subfamilies, Polistinae and Vespinae, share a common ancestor with the facultatively social Stenogastrinae. This monophyletic clade of three subfamilies, in turn, shares a common ancestor with the monophyletic Eumeninae, which contains the vast majority of solitary Vespidae as well as some communal and subsocial forms (Cowan 1991). If this phylogenetic hypothesis is true, then a social common ancestor of the three social subfamilies evolved from a solitary common ancestor shared with Eumeninae, and it did so while leaving no living forms that represent intermediate states of behavior, nesting, life history, or development between the solitary and social forms (Fig. 4). Diverse spe-

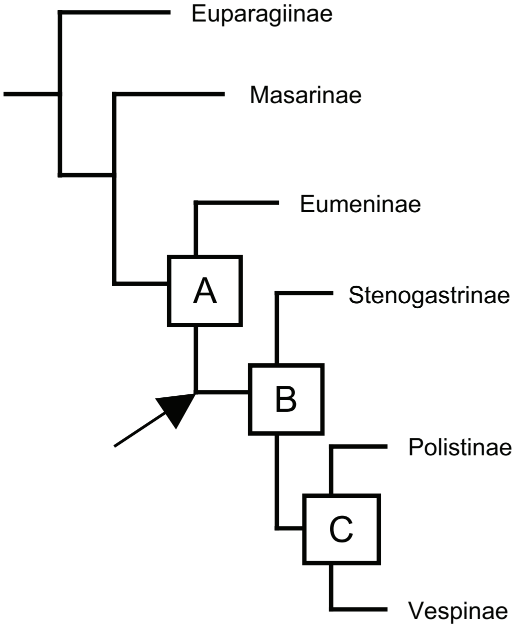

Fig. 4. The six-subfamily cladogram of relationships in Vespidae proposed by Carpenter (1982, 1989, 1991, 2003). Boxes A–C denote hypothetical common ancestors of subfamilies distal to each box. Subfamilies Polistinae and Vespinae contain exclusively eusocial forms, so their hypothetical common ancestor, C, would also have been eusocial. Stenogastrinae is considered to be eusocial or "facultatively eusocial" (e.g., Field *et al.* 1998), and so the hypothetical common ancestor B would also have been social, although the nature of that sociality cannot be strongly inferred. Because most Eumeninae are solitary (some are communal; some progressively provision larvae; none are eusocial, even facultatively so), hypothetical ancestor A would almost certainly have been solitary. Some eumenines (Miotk 1979, Bohart *et al.* 1982) have nesting biology and life history identical to that of the basal subfamily, Euparagiinae (Clement & Grissell 1968, Trostle & Torchio 1986), so a plausible inference is that hypothetical ancestor A possesses those traits: soil nesting and mass-provisioning with weevil larvae. Regardless of the details, however, the six-subfamily phylogeny necessitates the evolution of sociality, as would have been present in B, from solitary nesting, as would have been present in A, along the branch marked in the figure with an arrow. There are no living forms that represent intermediate states between A and B, according to this phylogeny. Eumeninae, however, contains many forms with traits that presage sociality: constructing complex nests above ground, use of plant material rather than mud for construction, a dietary shift to caterpillars, prey malaxation, progressive provisioning (reviewed in Hunt 2007). None of these traits and taxa can have any direct bearing on the origin of sociality, however, if Eumeninae is monophyletic. Instead, according to the six-subfamily phylogenetic hypothesis, sociality evolved from solitary ancestry (arrow) by means of changes in behavior and life history that cannot be inferred because of the total absence of living forms that represent intermediate stages.

cies of Eumeninae have seemingly informative behavioral and life history traits that have drawn the attention of numerous naturalists attempting to understand the evolution of vespid sociality (e.g., Roubaud 1916, Evans 1958, Evans & West-Eberhard 1970, West-Eberhard 1987c, Cowan 1991, Hunt 1999, 2007). However, these investigations cannot reveal ancestral states of sociality in the social subfamilies if Eumeninae is monophyletic. Faced with this conundrum, I reject monophyly of Eumeninae, and therefore the six subfamily phylogeny, as implausible. In addition to this line of argument based on salient life history features, there are specific and significant problems with the current data with which the six-subfamily phylogeny is supported. These are detailed in Hunt (2007) and include incorrect and inappropriate traits as putative synapomorphies of the social subfamilies, weakness of existing molecular data and analyses, and the near absence of phylogenetic treatment of the 3480 nominal forms in 211 genera of Eumeninae (numbers of taxa from J. M. Carpenter pers. comm.).

Levels of analysis

Proximate and *ultimate*, the terms most often used to frame levels of analysis debates, trace their roots to Mayr (1961). In the original example of bird migration (which I was taught, verbatim, in freshman zoology in the fall of 1962), physiological causes are labeled proximate, while genetic and ecological causes are labeled ultimate. Corollaries of the terms that have crept into popular consciousness are *mechanistic* and *evolutionary*. Despite the undeniable evolutionary nature of physiological (and all other) mechanisms, this dichotomy has become a cornerstone of behavioral ecology (e.g., Dugatkin 2004).

A behavioral ecologist challenged Amdam and me that by presenting only a mechanistic hypothesis we had committed a level of analysis error — that in order to understand the evolutionary reasons for altruistic behavior, one must consider kin-selected costs and benefits of working versus direct reproduction. However, we doubt that this approach has any greater potential for explanation than the framework we propose. For an offspring in a social wasp colony, working has costs (*c*) and benefits (*b*) that vary with relatedness (*r*) to reproductive kin according to Hamilton's rule, $rb - c > 0$. However, it has never been explicitly shown that social wasps can discriminate between nestmates on the basis of relatedness (Gamboa 2004). Mechanistically, then, *how* can costs and benefits provide an explanatory foundation for worker behavior? In the absence of data that elucidate how wasps assess costs and benefits as well as information that outlines how specific behaviors are based on these assessments, a cost-benefit explanation for the evolution of sociality can be as easily refuted, and by the same logic, as our challenger stated that he had refuted our hypothesis.

The levels of analysis challenge is based on a widespread and much more fundamental problem in the study of social insects, which is the precept that individual workers are the focus of selection (via inclusive fitness). West-Eberhard (1992: p. 73) puts social insect workers into a more appropriate focus: "*the castes of social insects — specialized workers and queens — are divergent but not dissociable: no one form* […] *can reproduce on its own. Rather, like the tissues and organs of a multicellular organism, they are mutually dependent on each other; like hands and feet, or liver and lungs, they are not readily dissociable*." In such a case, selection acts on the colony (Wilson & Hölldobler 2005, Hunt 2007). Wilson and Hölldobler (2005) discuss "the point of no return" in colony evolution. This is analogous to the threshold crossed in the evolution of multicellular individuality (Buss 1987, Maynard Smith & Szathmáry 1995). To take a clear example, no swarm-founding social insect species can ever revert to solitary life. Accordingly, the concept of colony-level selection must become as central and integral to the study of social taxa as the concept of individual-level selection is to multicellular organisms of diverse cell and tissue types. A colony of social insects is a remarkable level of evolutionary organization, the evolution of which is analogous to — and a step beyond — the evolution of multicellular individuality. We should recognize and study it as such.

Hypothesis testing as a way of knowing

With regard to the above-described contrasting ideas, to label one explanation unlikely to be true does not constitute support for a competing framework. Neither do hypotheses draw strong support from being elegant, intuitive, popular, or even, as is the case for the diapause ground plan hypothesis, because they have apparently high explanatory power. Hypotheses in science are best accepted only if they withstand rigorous tests of rejection (Platt 1964). Accordingly, the diapause ground plan hypothesis will stand or fall based on data that emerge from critical testing of its proposed components and of its predictions. The first tests have been completed of predictions made by Hunt and Amdam (2005: p. 266) concerning storage protein and development times in pupae (J. H. Hunt unpubl. data), and a genomics approach to testing the hypothesis of larval caste determination is under way (J. H. Hunt unpubl. data). In parallel with these efforts, several other lines of investigation should be undertaken.

Life cycles and reproductive ground plans of Eumeninae are precisely the places to look for an understanding of social evolution in Vespidae. One essential component of looking at eumenines, however, will be to look at them phylogenetically as part of a full survey of Vespidae based on well-conserved nuclear genes that can get at deep nodes in the clade. At present, GenBank houses taxonomically useful sequences of only two genes for only five species in only three genera of Eumeninae. Independent cladistic analyses of this information have led to radically different phylogenetic interpretations (Schmitz & Moritz 1998, Carpenter 2003), one of which differed significantly from phylogenetic inferences based on morphological characteristics. To assert that the available published data are robust and sufficient is an untenable position, and repeated reanalysis of the existing data is a waste of time. At the same time, to point out that the ancestor of *Polistes* was eusocial (hypothetical ancestor C in Fig. 4) does not invalidate the diapause ground plan hypothesis, which frames an approach to learning the nature of the solitary ancestor that preceded polistine eusociality. New molecular data are needed — from additional genes and with broad coverage of Eumeninae — and these data should be analyzed separately from existing data. Separate analysis of new molecular data will place the six subfamily hypothesis at risk of rejection and so would constitute a strong test of the six subfamily phylogeny.

A strong test of whether sociality evolved in a matrifilial or semisocial context could be a phylogenetic test — whether shared nesting or solitary nesting characterizes the non-social sister group to Polistinae + Vespinae. Such a test presupposes, however, that Eumeninae as currently recognized is paraphyletic with regard to the social subfamilies and that a sister taxon to Polistinae + Vespinae will be identified among living wasps, which can only be within Eumeninae.

West-Eberhard's ovarian ground plan hypothesis is based on partitioning of reproductive and foraging roles among nest sharing adults. Such behavioral and physiological partitioning may accurately describe the situation under which Sakagami and Maeta (1987a, 1987b) were able to artificially induce sociality by forcing nest sharing by two bees of the genus *Ceratina*, and it may apply in natural settings to sociality that arises among nest-sharing adults of a single generation in bees with small-colony sociality such as *Dialictus zephrum* (Michener 1969, 1974, 1990, Lin & Michener 1972). Interactions among adult nestmates clearly also lead to divergence of egg laying and foraging tasks among cofoundresses of a *Polistes* colony, and inter-adult interactions are proposed to affect the divergent ends of the non-diapause (G1) pathway in Fig. 2. None of these cases, however, constitute a test of the hypothesis that an ovarian cycle as described by West-Eberhard is the ground plan for worker/gyne caste partitioning among the offspring of a *Polistes* colony. One possible test would be to search for signatures of gene expression and hormone levels in solitary vespids that have ovarian cycles and then seek those same signatures in the worker and gyne castes of *Polistes* offspring. Candidate solitary vespids could be identified by the phylogenetic studies proposed above. In addition, West-Eberhard has very appropriately focused attention on the roles played by juvenile hormone in the partitioning of reproduction and working, but JH also will be integral to an understanding of the diapause ground plan hypothesis. Specific predictions in which JH data could potentially falsify one hypothesis or the other would provide a strong framework for advancement in this realm.

The hypothesis of altruism based on assessment of costs and benefits must be subjected to direct tests of potential mechanisms whereby individuals can assess and act on costs and benefits in circumstances in which assessment-based behavior options are available and can be expressed. It is important to note, however, that Hamilton's rule is not a population genetics model; it is a conceptual argument couched in algebraic terms that embodies an explanation for how selection can favor social behavior. It seems a simple-minded reading of Hamilton to think that variables of his famous inequality directly represent entities on which selection operates. As a conceptual framework, Hamilton's rule may be broadly applicable across social taxa, but mechanisms whereby Hamilton's rule is satisfied will be taxon-specific. To learn those mechanisms should be a primary goal for a broadly synthetic understanding of social evolution.

My colleague Patricia Parker placed the contests of ideas described here into a context I hereby explicitly adopt: "it is not a question of who is right; it is a question of getting it right." From this perspective, the way to address disagreements raised by the diapause ground plan hypothesis is not to assume an assertive stance in advocacy of a favored idea. The proper course — and the most rapid route to enlightenment — is to take each hypothesis and challenge it with rigorous tests of falsification. If the diapause ground plan hypothesis can be falsified, it will end up on the rubble heap. Current research in collaboration with Gro V. Amdam, Amy L. Toth, Gene E. Robinson, and other colleagues has been designed to take some predictions of the diapause ground plan hypothesis and directly test them as a potential falsification of the hypothesis. Other hypotheses should similarly be challenged with tests of refutation. A winnowing of hypotheses coupled with refinement of surviving hypotheses will be the result, and increased understanding will be the benefit.

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