Evolutionary Ecology Research, 2001, **3**: 331–344

Correlated evolution of colony defence and social structure: A comparative analysis in eusocial wasps (Hymenoptera: Vespidae)

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ABSTRACT

Animal societies depend on effective defence of group resources. Defensive mechanisms can be costly and may constrain the evolution of social structure. We analysed how exocrine mechanisms of colony defence were affected by the evolution of social complexity and of nest architecture in paper wasps (Vespidae). Eusocial paper wasp species exhibit two discrete grades of eusociality, with new colonies founded either by queens or by coordinated swarms of queens and workers. Swarm-founding shows multiple evolutionary origins from independent-founding ancestors within the Vespidae. Nest architecture also varies among paper wasps. Nests with covering envelopes evolved from naked combs several times. We hypothesized that: (1) evolutionary transitions from independent- to swarm-founding would obviate the need for chemical defence against ants and (2) transitions from naked combs to enveloped nests would have a similar effect on chemical defence. In support of the first hypothesis, we found that all independent-founding species possess ant-repellent glands (Van der Vecht's gland), while many swarm-founders do not. Furthermore, phylogenetic analysis suggested that evolutionary loss of this gland was statistically more likely to follow transitions to swarm-founding. Evolution of nest envelopes was less strongly associated with losses of the ant repellent gland. These patterns suggest that maintenance of defensive exocrine glands is costly. The patterns also suggest that group behavioural defence against ants is a key adaptive feature associated with the evolution of swarm-founding. The hypothesis that the evolution of nest envelopes obviated chemical defence against ants was not as well supported.

Keywords: ant predation, correlated evolution, independent-founding, social behaviour, swarm-founding, sternal glands.

INTRODUCTION

Living in social groups imposes a number of costs on members (Alexander, 1974). For example, many animal societies accumulate resources (brood and food stores) that attract

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conspecific and heterospecific natural enemies. These resources must be defended, and defence incurs some cost to the society. Recent theory suggests that defensive adaptations correspond to evolutionary transitions among different social systems (Starr, 1985; Costa and Pierce, 1997; Crespi and Choe, 1997; Wolff and Peterson, 1998; Hunt, 1999). Comparative analyses can provide empirical tests for correlated evolution of defence and social systems. We performed a test for correlated evolution of social structure and defence in paper wasps (Vespidae) that exhibit different grades of eusociality. Our aim was to quantify the relationships of evolutionary transitions in mode of colony foundation and in nest architecture with the evolution of exocrine defensive mechanisms.

Paper wasps exhibit two grades of eusociality, according to whether colonies are initiated by queens (independent-founding species) or by groups of queens and workers (swarm-founding species). Parsimony suggests that swarm-founding behaviour evolved four times from independent-founding ancestors in the Vespidae (Carpenter, 1991; Wenzel and Carpenter, 1994). Nest-covering envelopes also arose several times, each time from stalked, open-combed nests (Jeanne, 1975; Wenzel, 1991, 1993).

Ants are important natural enemies of eusocial wasps and represent a major selective force on wasp social and defensive behaviour (Jeanne, 1975, 1979; Chadab, 1979; Kojima, 1993). Ants that locate wasp nests are usually detected and removed. Because independently founded nests are left unattended during foraging, they are vulnerable to predation by ants. All independent-founding species of Vespidae studied to date use exocrine secretions for nest defence against ants. The glandular source of the ant repellent is Van der Vecht's gland, located on the sixth sternite of the gaster (Van der Vecht, 1968; Jeanne, 1970, 1996; Turillazzi and Ugolini, 1979; Post and Jeanne, 1981; Kojima, 1983, 1992; Keeping, 1990).

In contrast to independent-founders, swarm-founding species never leave new nest sites unattended. We hypothesized that ant repellent secretions are unnecessary in swarm-founding species, because the wasps in a swarm are always present on the nest to physically defend against intruders (Jeanne *et al.*, 1983; West-Eberhard, 1989; O'Donnell and Jeanne, 1990). We assume that ant predation was an important selective force on all species of Vespidae, and we use the presence or absence of Van der Vecht's gland to indicate evolutionary changes in ant defence strategies. If the glands are costly to produce or maintain, we predict that they would be lost in lineages that evolved swarm-founding. We used the well-resolved phylogeny of eusocial Vespidae (Vespinae + Polistinae; Wenzel and Carpenter, 1994) to statistically test evolutionary hypotheses of the association of mode of colony founding with Van der Vecht's gland.

We also tested whether the origin of nest envelopes was correlated with the evolution of exocrine nest defence. Nest envelopes may also serve as a defence against insect predators such as ants (Jeanne, 1975). All known independent-founding wasps in the subfamily Polistinae have similar nest architecture: they suspend uncovered nest combs from a thin petiole (Wenzel, 1991, 1993). Adult females rub the petiole with the ant repellent secretion of Van der Vecht's gland. Most swarm-founding Polistinae, and all species of the subfamily Vespinae (yellowjackets and hornets, the sister clade to the Polistinae), build an envelope around their nest combs (Wenzel, 1991, 1993). A nest envelope may serve as a physical defence against ants by restricting access to the combs and by providing a defendable entryway (Jeanne, 1975). Like behavioural defence against ants by the workers in a swarm, a nest envelope may render the ant-repellent secretion of Van der Vecht's gland unnecessary (London and Jeanne, 2000). If this is the case, we predict that the glandular source of ant repellent would be more likely to be lost in lineages with a nest envelope than in those that build open nests.

Jeanne *et al.* (1983) surveyed the gland morphology and nest-founding behaviour of 62 species of Vespidae. Their sample included 24 of the 27 genera of Polistinae, but a phylogeny of intergeneric relationships in Vespidae was not available. Without examining behaviour and gland possession in a phylogenetic framework, it is impossible to discern whether apparent associations are due to shared evolutionary history (i.e. closely related species share states of both characters) or to the functional features of the traits (i.e. certain social structures require certain defensive mechanisms; Felsenstein, 1985; Harvey and Pagel, 1991; Martins and Hansen, 1996). For example, all but four of the 47 swarm-founding species surveyed by Jeanne *et al.* belong to a single clade, the Neotropical tribe Epiponini. Thus, it was difficult to distinguish between traits characteristic of all swarm-founders from those characteristic only of epiponines.

In this study, we extend the exocrine gland survey of Jeanne *et al.* (1983) and use the data on the presence or absence of Van der Vecht's gland for comparative tests of correlated evolution. We determine gland morphology for most of the polistine genera not included in the original survey, and broaden the scope of the survey to include the subfamily Vespinae. We map sternal gland morphology, mode of colony founding and nest architecture of all genera for which data were available onto the cladogram of the Vespinae + Polistinae (Carpenter, 1991; Wenzel and Carpenter, 1994). Where possible, we use the concentrated changes test (Maddison, 1990) to statistically analyse the following evolutionary predictions: (1) species in independent-founding lineages will possess Van der Vecht's gland (the source of ant repellent); (2) the loss of Van der Vecht's gland is more likely to occur in swarm-founding lineages, which may no longer require an ant repellent secretion, than in independent-founding lineages; (3) the loss of Van der Vecht's gland is more likely to occur in lineages with a nest envelope. We conclude by discussing the implications of our findings for the evolution of swarm-founding behaviour, social structure and colony defence.

METHODS

Histology

We obtained wasp specimens from colleagues and from our own collections. Adult female workers were collected from nests or by netting foragers. Wasps were placed directly into fixative or preservative in the field (Kahle's solution, 18 : 1 : 1 volumes of 70% ethanol : formalin : glacial acetic acid; or 70% ethanol). We dissected out the sixth sternite (Van der Vecht's gland, ant repellent source) and embedded it in resin (Spurr, 1969). Thin sections (2 μm) were cut along a sagittal plane with a glass knife on a Leica Ultracut R microtome. We mounted the sections on gelatin-coated slides, stained with toluidine blue, and examined them with a light microscope at between 100 and 1000 power magnification depending on the size of the sternite.

Our histological sample included 19 species. Our sample increased the number of histologically examined species from one to two for nine genera and species groups, and we sampled seven genera and species groups that had not been examined previously. We assayed gland morphology for one to three specimens for each species. We saw no evidence of intraspecific variation in gland morphology; intraspecific variation in gland possession was not noted in previous studies (Jeanne *et al.*, 1983). Each species was scored as possessing or not possessing the sixth sternal gland, based on whether class III gland cells were associated with the anterior margins of the sternites (Fig. 1; Hermann and Dirks, 1974; Noirot and Quennedey, 1974, 1991; Jeanne and Post, 1982; Jeanne *et al.*, 1983; Keeping, 1990; Downing, 1991; Raposo-Filho *et al.*, 1994). Gland data for two species of *Polybioides* were provided by S. Turillazzi (personal communication). For the remaining taxa, we used previously published gland morphology data (Table 1).

Behavioural data

We determined whether a species was independent-founding or swarm-founding from published accounts and from our own observations. For some species, nest-founding swarms have been observed in transit or in the process of new nest construction. For other species, evidence for swarm-founding is indirect. Initial colony sizes above approximately 50 adults gave indirect evidence for swarming (Jeanne *et al.*, 1983). Data on nest architecture, such as the presence or absence of nest envelopes, were obtained from the literature and from our own observations (Jeanne, 1975; Wenzel, 1991).

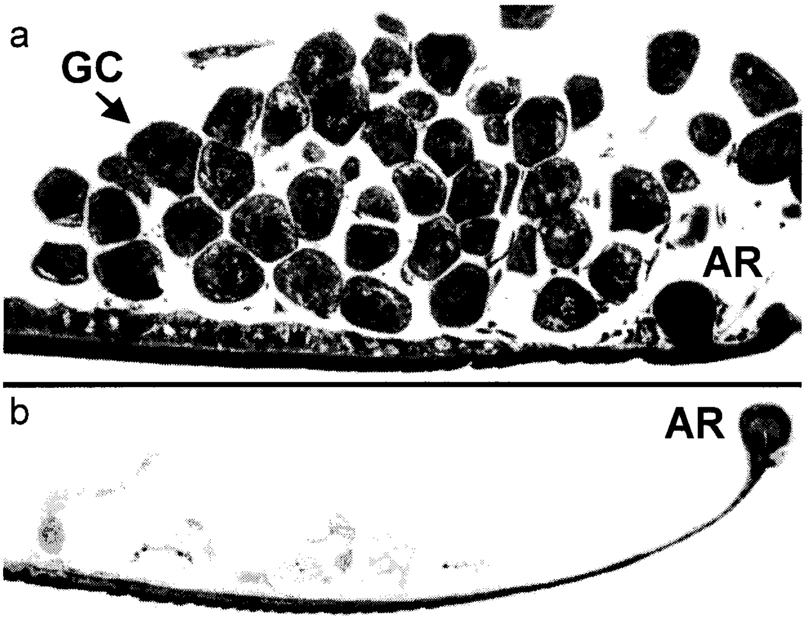

Fig. 1. Longitudinal section of the anterior area of the sixth sternite (100× magnification). Anterior is at right. The sternite cuticle is at the base of each photograph. AR indicates the anterior ridge of the sternite. GC indicates a representative gland cell. (a) *Dolichovespula maculata*, an example of a species with Van der Vecht's gland. (b) *Chartergellus punctatior*, an example of a species without Van der Vecht's gland.

Phylogenetic analyses

All phylogenetic analyses were based on a fully resolved cladogram of genera of Vespidae (approximate analysis of 97 behavioural and morphological characters led to 41 cladograms with length 314, consistency index 0.48 and retention index 0.70; a single fully resolved cladogram resulted from successive weighting: Carpenter, 1991; Wenzel and Carpenter, 1994) with modifications as follows. When cladograms were available to resolve species at higher resolution than genera (species groups in *Vespula* and *Ropalidia*), we used the highest resolution available. Carpenter *et al.* (1996) synonymized the vespid genera *Occipitalia* with *Clypearia*. J. Kojima (personal communication) provided preliminary data on relationships among *Ropalidia* species groups. Because Richards' (1978) *Ropalidia* subgenera are ill-defined (Kojima, 1997a,b; Kojima and Carpenter, 1997), we used Kojima's (personal communication) preliminary *Ropalidia* species groups. Neither the original cladograms nor subsequent modifications provided bootstrap values for the phylogeny.

We included data from three of the six genera of the subfamily Stenogastrinae (hover wasps; Carpenter, 1988) to infer the ancestral states of characters for the Vespinae + Polistinae. Carpenter (1991) placed Stenogastrinae as the sister subfamily to Vespinae + Polistinae (Vespidae). Schmitz and Moritz (1998) argue that Stenogastrinae is not the sister clade to Vespinae + Polistinae, but this does not change its placement as an outgroup for our analysis. Schmitz and Moritz (1998) also presented molecular evidence that *Provespa* is the sister genus to *Vespa,* rather than to *Dolichovespula* + *Vespula*. Because this minor change does not affect our analysis, and is the only distinction between Schmitz and Moritz (1998) and the more complete phylogeny of Wenzel and Carpenter (1994), we confined our analysis to the latter.

We mapped character states onto the cladogram and we inferred ancestral states using unordered parsimony as implemented by MacClade version 3.01 (Maddison and Maddison, 1992). Similar patterns of evolutionary transitions for two characters across independent lineages were taken as evidence of an association between traits (Felsenstein, 1985; Harvey and Pagel, 1991). Testing whether such patterns are statistically significant requires specialized procedures, due to the non-independence of data points caused by phylogenetic history (Felsenstein, 1985; Martins and Hansen, 1996; see Wenzel and Carpenter, 1994, for alternative views on the use of phylogeny in comparative analyses). We employed the concentrated changes test (Maddison, 1990) to determine whether evolutionary changes in gland presence are significantly concentrated in lineages with different modes of colony founding or nest architecture, given the most parsimonious reconstruction of both characters' evolution. The concentrated changes test (Maddison, 1990) was performed using either the 'exact count' or 'simulations' ($n = 10{,}000$) options in MacClade (Maddison and Maddison, 1992). Simulation runs were performed when we analysed the entire vespid phylogeny, which was too large for exact count analysis. We used exact count analysis when testing patterns on collapsed cladograms (see section on 'Loss of Van der Vecht's gland and swarm-founding'). We checked agreement of these methods by also performing simulation runs on all collapsed trees; estimated P-values always agreed within ± 0.01.

The concentrated changes test cannot be used to analyse unresolved nodes (polytomies; Maddison, 1990), so our analysis included no more than two species for the highest resolved clade (genus or species group). Additional data on gland morphology were available for seven genera or species groups (from three to five species sampled per group). Because we found no intra-generic or intra-species group variation in gland possession (Table 1), only

Table 1. Glands of sixth sternites in female stenogastrine, vespine and polistine wasps

Species	F	6	E	Reference
Stenogastrinae				
Liostenogaster vechti	S*i*	–	–	11
Parischnogaster striulata	I	–	–	11
Stenogaster mellyi	I	–*s*	–	1
S. iridipennis	I	–*s*	–	1
Vespinae				
Vespa crabo	I	+	+	3
V. orientalis	I	+	+	3
Provespa nocturna	S	+	+	11
P. anomala	S	+*s*	+	1
Dolichovespula arctica	I	+	+	3
D. arenaria	I	+	+	3
D. maculata	I	+	+	3, 11
Vespula vulgaris (*vulgaris* group)	I	+	+	3
V. germanica (*vulgaris* group)	I	+	+	3
V. pennsylvanica (*vulgaris* group)	I	+	+	3
V. maculifrons (*vulgaris* group)	I	+	+	3
V. atropilosa (*rufa* group)	I	+	+	3
V. acadica (*rufa* group)	I	+	+	3
V. vidua (*rufa* group)	I	+	+	3
V. squamosa (*squamosa* group)	I	+	+	3
Polistinae				
Polistes fuscatus	I	+	–	3, 5, 7
P. canadensis	I	+	–	5, 7
P. annularis	I	+	–	2, 7
P. gallicus	I	+	–	4
P. foederatus	I	+	–	4
Mischocyttarus flavitarsis	I	+	–	3, 7
M. mexicanus	I	+	–	7
M. atramentarius	I	+	–	9
M. immarginatus	I	+	–	7
Polybioides tabidus	S	–	+	10
P. raphigastra	S	–*s*	+	10
Belonogaster grisea	I	+	–	7
B. petiolata	I	+	–	8
Ropalidia horni	I	+	–	7
R. cincta	I	+	–	11
R. fasciata	I	+	–	7
R. romandi	S	–	+	11
R. extrema (*flavopicta* group)	S*i*	–	+	7
R. montana (*flavopicta* group)	S*i*	–	+	7
R. nigrescens (*flavopicta* group)	S*i*	–	–	7
R. flavobrunnea (*flavopicta* group)	S*i*	–	–	7
Parapolybia indica	I	+	+	7
P. varia	I	+	+	7
Apoica pallida	S	+	+	7
A. flavissima	S	+	+	7
Angiopolybia pallens	S	–	–	7
Agelaia = (*Stelopoybia*) *areata*	S	+	–	7

A. testacea	S	+	−	7
A. panamaensis	S	+	−	7
A. myrmecophila	S	+	−	7
Pseudopolybia compressa	S	−	+	7
P. difficilis	S	−	+	7
Parachartergus fraternus	S	−	+	7
P. colobopterus	S	−	+	11
Leipomeles nana	S	−	+	11
L. dorsata	S	−	+	7
Chartergellus punctatior	S	−	+	11
C. communis	S	−*s*	+	7
Nectarinella championi	S	−	+	11
N. xavantina	S	−	+	11
Synoeca virginea	S	−*s*	+	7
S. surinama	S	−	+	7
Clypearia apicipennis	S*i*	−	+	7
C. (= *Occipitalia*) *sulcata*	S	−	+	7
Metapolybia docilis	S	−	+	7
M. cingulata	S	−	+	11
Protopolybia exigua	S	−	+	11
P. alvarengai	S	−*s*	+	7
Charterginus fulvus	S	−	+	11
Chartergus metanotalis	S*i*	−	+	11
C. globiventris	S*i*	−	+	11
Brachygastra augusti	S	−	+	7
B. smithii	S	−	+	7
B. lecheguana	S	−	+	7
B. bilineolata	S	−	+	11
Protonectarina sylverirae	S	−	+	11
Polybia sericea	S	−	+	6
P. chrysothorax	S	−	+	6
P. occidentalis	S	−	+	6
P. quadricincta	S	−	+	6
Epipona guerini	S	−	+	11
E. tatua	S	−*s*	+	7

Note: Genera are ordered vertically corresponding to the order in Figs 2 and 3. All species with histological data are included. Species in which the presence or absence of Van der Vecht's Gland is inferred from cuticular morphology (Jeanne *et al.*, 1983) are included if no histological data, or histological data from only one other congener, are available. Where surface data contradict histological data, the histological data are used for analysis. Column 'F' indicates founding type: S = swarm, I = independent, S*i* = evidence of swarming is indirect. Column '6' refers to the sixth sternite: '+' indicates presence of gland, '−' indicates absence, *s* indicates that presence or absence is inferred from the surface morphology of the cuticle (no histology), '?' indicates unknown. Column 'E' lists the presence (+) or absence (−) of a nest envelope (from Wenzel, 1991, 1993). References: 1 = Van der Vecht (1968), 2 = Hermann and Dirks (1974), 3 = Landolt and Akre (1979), 4 = Turillazzi (1979), 5 = Post and Jeanne (1980), 6 = Jeanne and Post (1982), 7 = Jeanne *et al.* (1983), 8 = Keeping (1990), 9 = Raposo-Filho *et al.* (1994), 10 = S. Turillazzi (personal communication), 11 = present study.

genera or species groups are shown in our figures. However, all analyses were based on the highest possible phylogenetic resolution: two representative species of each clade, except that nine of the 31 vespid genera and species groups were represented by histologically sectioned specimens of only one species (one genus, *Protonectarina*, is monotypic; details in Table 1). We could not obtain specimens of the epiponine genera *Asteloeca*, *Marimbonda* and *Synoecoides*, and of the *Vespula* species group *koreensis*.

RESULTS

Independent-founding and possession of Van der Vecht's gland

Independent-founding and possession of Van der Vecht's gland were both inferred to be ancestral for the Vespinae + Polistinae (Fig. 2). Van der Vecht's gland was absent in the Stenogastrinae (Table 1), so one gain of each character was present (at the base of the Vespinae + Polistinae clade). Within the Vespinae + Polistinae, every independent-founding species had Van der Vecht's gland (Table 1).

Loss of Van der Vecht's gland and swarm-founding

All four changes in Van der Vecht's gland character state occurred on swarm-founding branches (Fig. 2). There are two equally parsimonious reconstructions of the evolution of Van der Vecht's gland. In one, the gland was lost on the branch leading to *Angiopolybia* and

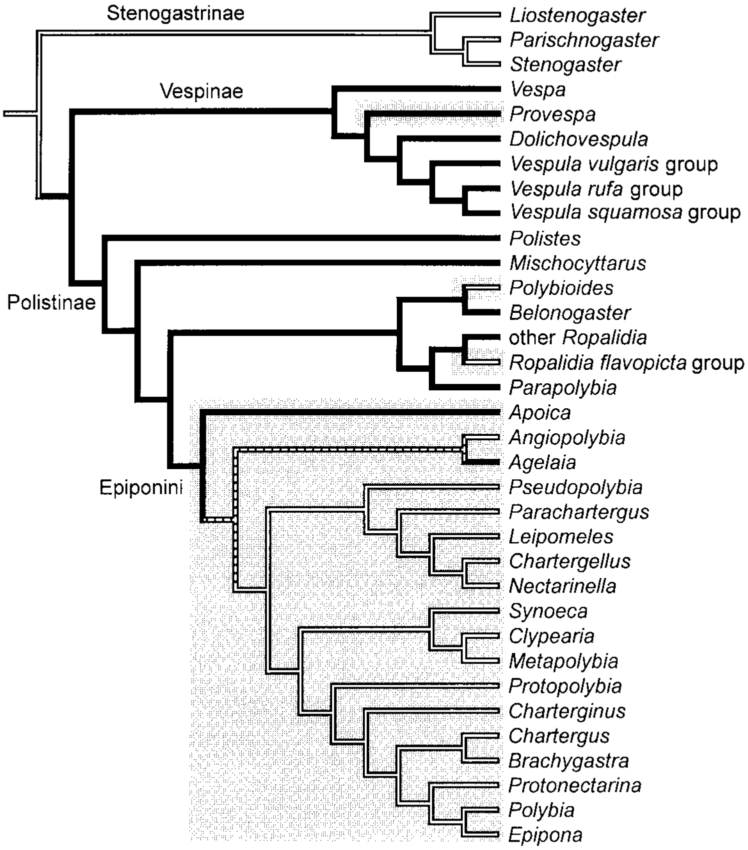

Fig. 2. Evolution of Van der Vecht's gland and nest-founding behaviour in the Vespinae + Polistinae. The presence of Van der Vecht's gland is represented by black, its absence by white and uncertainty by striped branches. Swarm-founding lineages are on shaded background. Van der Vecht's gland has been lost three or four times, all on swarm-founding branches.

again on the branch leading to all species derived after the *Angiopolybia* + *Agelaia* divergence. Assuming that all gains and losses are equally likely, it is equally parsimonious for the gland to be lost before the *Angiopolybia* + *Agelaia* divergence, and then regained by *Agelaia* (Fig. 2).

Although all losses (either three or four) of Van der Vecht's gland occurred on swarm-founding branches, this association was not significant when tested on the full cladogram (concentrated changes test: $P = 0.10$ for four losses, $P = 0.18$ for three losses). However, this P-value was heavily influenced by the topology of the tree (Maddison, 1990; Sillén-Tullberg, 1993; Read and Nee, 1995). When all clades in which Van der Vecht's gland has already been lost were collapsed, thus preventing these lineages from being counted as further opportunities for loss, Van der Vecht's gland was significantly more likely to be lost in swarm-founding lineages than in independent-founding lineages (four losses: $P = 0.005$; three losses: $P = 0.02$).

Loss of Van der Vecht's gland and nest architecture

All vespines examined to date build nest envelopes (Wenzel, 1991, 1993) and possess Van der Vecht's gland (Table 1). Within the Polistinae there have been three or four losses of Van der Vecht's gland (see above), between two and four of which occurred on envelope-building lineages (Fig. 3). There are two equally parsimonious reconstructions of the evolution of a nest envelope (Fig. 3). In one, the trait arose at the base of all epiponines derived after *Apoica*, and was lost in *Agelaia*. In the other reconstruction, nest envelopes evolve independently in *Angiopolybia* and the branch leading to all species derived after the *Angiopolybia* + *Agelaia* divergence. Because reconstruction of the evolution of Van der Vecht's gland was uncertain on the same branches (see above), it is possible that four of four, three of four, three of three, or two of three losses of Van der Vecht's gland occurred in envelope-building lineages (Fig. 3). In none of these scenarios was Van der Vecht's gland significantly more likely than chance to be lost on branches of envelope-building lineages (concentrated changes test: $P = 0.22$ to $P = 0.47$). However, when the clades in which Van der Vecht's gland has already been lost were collapsed under the assumptions discussed above, four of four ($P = 0.04$), but not fewer, losses of Van der Vecht's gland in envelope-building lineages ($P = 0.08$ to $P = 0.41$) are significantly more than expected by chance. The character state of nest envelopes is equivocal for the genus *Agelaia*, because at least two species of *Agelaia* (*A. areata* and *A. flavipennis*) build envelopes (Wenzel, 1998). Lack of envelopes in most cavity-dwelling *Agelaia* species is probably derived from envelope-building in the genus (Wenzel, 1991). Treating *Agelaia* as an envelope-building lineage yields similar results to those above for both the uncollapsed ($P = 0.28$) and collapsed trees ($P = 0.08$).

DISCUSSION

Our data suggest that defence against ants was affected by the evolution of social structure in eusocial Vespidae. The ability to produce ant repellent is a key adaptation for independent-founding wasps. All independent-founding vespines and polistines possess Van der Vecht's gland. In all independent-founding genera in which it has been tested, Van der Vecht's gland secretion repels ants (Jeanne, 1970; Turillazzi and Ugolini, 1979; Post and

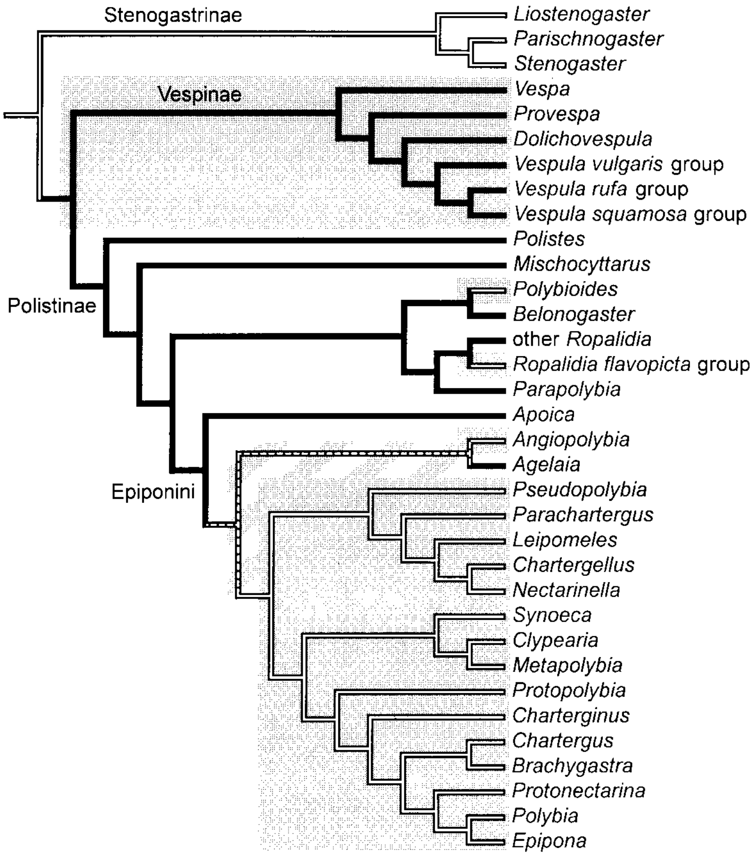

Fig. 3. Evolution of Van der Vecht's gland and nest architecture in the Vespinae + Polistinae. The presence of Van der Vecht's gland is represented by black, its absence by white and uncertainty by striped branches. Lineages that build nest envelopes are on shaded background. Intermittent shading represents uncertainty in the nest architecture character state. There have been three or four losses of Van der Vecht's gland, between two and four of which occurred in envelope-building lineages.

Jeanne, 1981; Kojima, 1983, 1992; Keeping, 1990). Repellency of the secretions has not been tested in the Vespinae (Matsuura and Yamane, 1984). The thin petiole from which independent-founding polistines hang their nest enhances the effectiveness of the ant repellent by concentrating it on a small area (Jeanne, 1975). Stenogastrine wasps, the putative sister taxon of Vespinae + Polistinae (Carpenter, 1991), lack Van der Vecht's gland. Stenogastrines produce an ant repellent secretion from a different exocrine gland, Dufour's gland (Turillazzi and Pardi, 1981; Turillazzi, 1985).

Because swarm-founding allows wasps to leave guards at new nests, swarm-founding species should not require the ant repellent secretion of Van der Vecht's gland. In every swarm-founding lineage where the sixth sternal gland has been studied, either the gland or the ant repellent function of its secretion has been lost. Only three of 21 swarm-founding genera possess Van der Vecht's gland. In two of these genera (*Agelaia* and *Apoica*), Van der Vecht's gland secretion does not repel ants (London and Jeanne, 2000), while the third genus (the vespine *Provespa*) has not been tested. The function of the gland in these three genera is unknown (Jeanne *et al.*, 1983; Hunt *et al.*, 1995).

All evolutionary changes in the possession of Van der Vecht's gland occurred in swarm-founding lineages, involving either three losses and one gain (in *Agelaia*), or four losses of the gland. Given that there are no additional cases of the reappearance of Van der Vecht's gland after its loss, we believe that it is unlikely that *Agelaia* would regain Van der Vecht's gland following evolutionary loss. The available phylogeny cannot be further resolved to distinguish between these two equally parsimonious scenarios. However, regardless of the evolutionary history of the gland, *Agelaia* has lost its ancestral ant repellent function (London and Jeanne, 2000).

In our study, the concentrated changes test answers the question, 'Given that there are four losses of Van der Vecht's gland, what is the expected probability of all four occurring in swarm-founding lineages?' Because 21 of the 31 genera or species groups in our study are swarm-founding, the chance occurrence of all losses on swarm-founding branches is possible. Yet examination of Fig. 2 shows that Van der Vecht's gland was lost before either 16 or 17 of the 21 swarm-founding genera or species groups evolved. Thus, many of the branches on the full tree are in clades in which the gland had already been lost. The number of taxa in a phylogenetic analysis can influence the outcome of the concentrated changes test, even if additional clades do not exhibit gains or losses of the traits being analysed (Maddison, 1990; Sillén-Tullburg, 1993; Read and Nee, 1995). Because the many swarm-founding branches in which Van der Vecht's gland has already been lost cannot lose the gland again, their inclusion inflates the probability of Type II error (failing to reject the null hypothesis). This is why we collapsed these branches of the tree for the above analyses. Under the assumption that secondary evolutionary gain and subsequent loss of sternal glands is unlikely, we found evidence for a significant association between swarm-founding and loss of Van der Vecht's gland.

As most swarm-founders also build enclosed nests, it could be argued that the nest envelope protects against ants (Jeanne, 1975), allowing the evolutionary loss of an ant repellent secretion. However, all vespines build enclosed nests (Wenzel, 1991, 1993), yet all have also retained Van der Vecht's gland (although ant repellent ability has not been tested in vespines). The two epiponine swarm-founding genera without nest envelopes (*Apoica* and most *Agelaia*) are the only ones that retained Van der Vecht's gland, but the gland's ant repellency has been lost (London and Jeanne, 2000). Although nest envelopes are undoubtedly important in colony defence (Jeanne, 1975), our data suggest that their presence or absence is less informative than the mode of colony founding for explaining patterns of loss of Van der Vecht's gland.

Jeanne (1991) proposed that improved defence against ants is a major adaptive benefit resulting from the evolution of swarm-founding. Our results support this hypothesis. Every swarm-founding species, and no independent-founding species, is known to have lost either Van der Vecht's gland or its ant repellent function. This pattern is extremely unlikely unless swarm-founding enables improved ant defence by other mechanisms (Jeanne, 1991). The evidence suggests that chemical ant repellent is a key adaptation for independent-founding wasps (Jeanne, 1970; Turillazzi and Ugolini, 1979; Post and Jeanne, 1981; Kojima, 1983, 1992; Keeping, 1990). This taxon-wide, ecologically important defensive mechanism has apparently been rendered unnecessary in every case of repeated evolutionary origins of advanced social structure.

ACKNOWLEDGEMENTS

We thank J.M. Carpenter, J.H. Hunt, M.G. Keeping, J. Kojima, C. Nalepa and F.B. Noll for contributing specimens, and F.R. Dani, J. Kojima and S. Turillazzi for sharing unpublished data. T.A. Jones generously gave advice on histological techniques and access to her laboratory facilities. Emília Martins, John Wenzel and two anonymous reviewers made helpful comments on previous drafts of the manuscript. Financial support was provided by grants from the National Science Foundation (to R.L.J. and to S.O'D.) and the University of Washington Royalty Research Fund (to S.O'D.).

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