Morphological and physiological variation between queens and workers of *Protonectarina sylveirae* (de Saussure) (Hymenoptera, Vespidae, Epiponini)

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ABSTRACT. Morphological and physiological variation between queens and workers of *Protonectarina sylveirae* (de Saussure) (Hymenoptera, Vespidae, Epiponini). The Neotropical swarm-founding wasps, Epiponini, range from the absence of morphological differentiation between castes to highly distinct castes. We measured eight body parts of females of two colonies of *Protonectarina sylveirae* (de Saussure, 1854). ANOVA and Discriminant Analysis evidenced significant differences between castes, as previously observed by other authors for other species of Epiponini. However, some females previously categorized as queens, were actually workers, supported by our statistic analyses. These individuals showed intermediate morphological features between queens and workers, having distinct patterns of hairs and clypeal spots. The castes of *P. sylveirae* are distinct, however intermediate individuals may be found in colonies promoting social flexibility.

KEYWORDS. caste differentiation; Polistinae; social wasps.

RESUMO. Variações morfológicas e fisiológicas entre rainhas e operárias de *Protonectarina sylveirae* (de Saussure) (Hymenoptera, Vespidae, Epiponini). As vespas enxameadoras neotropicais, Epiponini, apresentam desde ausência de diferenciação morfológica entre as castas até castas altamente distintas. Medimos oito partes corporais das fêmeas de duas colônias de *Protonectarina sylveirae* (de Saussure, 1854). ANOVA e Análise Discriminante evidenciaram diferenças significativas entre as castas, como observado anteriormente por outros autores em outros Epiponini. No entanto, algumas fêmeas, previamente categorizadas como rainhas, eram na verdade operárias, com base nas análises estatísticas. Esses indivíduos apresentaram características morfológicas intermediárias entre rainhas e operárias, tendo padrões distintos de pilosidade e da marca do clípeo. As castas em *P. sylveirae* são distintas, no entanto indivíduos intermediários podem ser encontrados nas colônias promovendo uma flexibilidade social.

PALAVRAS-CHAVE. diferenciação de castas; Polistinae; vespas sociais.

The Neotropical swarm-founding wasps, Epiponini, are characterized by the occurrence of polygyny, complexity of nest construction and slight differentiation between queens and workers (Richards & Richards 1951; Richards, 1971, 1978). Different patterns of caste differentiation can be found in species of Epiponini (Richards & Richards 1951; Jeanne & Fagen, 1974; Noll & Wenzel, 2008) such as: 1) no morphological differences between queens or workers, found in *Angiopolybia pallens* (Lepeletier, 1836) (Richards 1978, Noll *et al*. 2004); *Clypearia sulcata* (Saussure, 1854) (Noll *et al*. 2004); *Leipomeles dorsata* (Fabricius, 1804) (Noll *et al*. 2004); *Metapolybia aztecoides* Richards, 1978 (Noll *et al*. 2004); *Nectarinella championi* (Dover, 1925) (Noll *et al*. 2004); *Synoeca surinama* (Linnaeus, 1767) (Richards 1978; Noll *et al*. 2004); 2) queens larger than workers and intermediates found in *Chartergus metanotalis* Richards, 1978 (Noll *et al*. 2004); *Epipona tatua* (Cuvier, 1797) (Richards, 1978; Noll *et al*. 2004); *Polybia liliacea* (Fabricius, 1804) (Noll *et al*. 2004); *P. rejecta* (Fabricius, 1798) (Richards 1978; Noll *et al*. 2004); *P. spinifex* Richards, 1978 (Noll *et al*. 2004); 3) females with ovary developed but with spermatheca empty, found in *Brachygastra scutellaris* (Fabricius, 1804) (Richards, 1978); *Polybia bistriata* (Fabricius, 1804) (Richards & Richards 1951; Richards 1978); *P. emaciata* Lucas, 1879 (Richards & Richards 1951; Richards 1978); *P. occidentalis* (Olivier, 1791) (Richards & Richards 1951; Richards 1978); *P. paulista* (von Ihering, 1896) (Noll *et al*. 1996); *Parachartergus smithii* (de Saussure, 1854) (Mateus *et al*. 1997); *Protopolybia exigua* (de Saussure, 1854) (Noll *et al*. 1996); *Psudopolybia vespiceps* (de Saussure, 1864) (Shima *et al*. 1998); and 4) queens with smaller heads and larger abdomens than workers, found in *Apoica flavissima* Van der Vecht, 1973 (Shima *et al*. 1994); *Agelaia multipicta* (Haliday, 1836) (Noll *et al*. 1997); *A. pallipes* (Olivier, 1791) (Richards 1978; Noll *et al*. 1997); *A. vicina* (de Saussure, 1854) (Baio *et al*. 1998).

According to Shima *et al*. (1996, 2003), *Protonectarina sylveirae* is similar to other species of Epiponini which show diphasic allometry (caste differentiation occurs by differentiation on shape and size) denoting pre-imaginal caste determination (Noll *et al.* 2004).

The monotypic genus *Protonectarina* is widely distributed, from Brazil to Argentina and can be diagnosed by the width clypeus, the hairy eyes and the tergum I not petiolate in dorsal view (Richards 1978; Carpenter & Marques 2001). Nests are arboreal, usually suspended from a twig (Richards 1978; Wenzel 1998). The primary envelope is built from substrate and the secondary from previous envelope or partly from

Table I. Means in millimeters (± SD) and observed values of ANOVA test, for eight characters used for discriminating castes of colonies of *P. sylveirae*.

		Means ± SD			
Colony #	Characters	Queens	Workers	ANOVA	p
I		n=260	n=50	(F)	
	HW	2.04 ± 0.06	2.10 ± 0.07	41.10	<0.01
	Pml	1.00 ± 0.03	0.96 ± 0.04	41.29	<0.01
	MSW	1.82 ± 0.07	1.77 ± 0.09	17.77	<0.01
	T_1BW	1.37 ± 0.07	1.12 ± 0.07	509.65	<0.01
	AL	3.13 ± 0.11	2.99 ± 0.14	60.40	<0.01
	T_1BH	0.31 ± 0.04	0.28 ± 0.05	16.54	<0.01
	T_1L	1.44 ± 0.07	1.30 ± 0.11	131.04	<0.01
	WL	3.04 ± 0.10	2.91 ± 0.15	59.33	<0.01
II		n=500	n=50	(F)	
	HW	2.01 ± 0.06	2.08 ± 0.08	58.79	<0.01
	Pml	0.98 ± 0.03	0.97 ± 0.04	11.86	<0.01
	MSW	1.77 ± 0.07	1.79 ± 0.07	1.70	0.19
	T_1BW	1.30 ± 0.08	1.16 ± 0.07	162.68	<0.01
	AL	3.08 ± 0.11	3.01 ± 0.14	15.61	<0.01
	T_1BH	0.26 ± 0.05	0.30 ± 0.03	25.87	<0.01
	T_1L	1.37 ± 0.08	1.32 ± 0.09	11.63	<0.01
	WL	2.95 ± 0.11	2.90 ± 0.11	9.46	<0.01

substrate, seams marking successive envelopes sinuous and irregular; the entrance is simple, in ventral surface of nest, aligned from internal passage between stories (Wenzel 1998). The nests may contain large populations and amounts of nectar (Richards 1978; Wenzel 1998; Shima *et al.* 2003).

According Shima *et al.* (1996), queens show lighter body color and less contrast among colors in comparison with workers. Clypeus region in queens follows a pattern uniformly wide, ventrally emarginated and with a brown spot. Among workers this region has a black spot horseshoe-shape with great variation (Fig. 1). Besides that, there is some variation in the punctures of the second gastral tergite which is larger in workers than in queens (Richards 1978). An interesting aspect found by Shima *et al*. (1996) was a large variation among queens' size, so some queens may be as smaller as workers, even though morphological characters may distinct them.

The nests are large, either cylindrical or expanding downwards up to 30-40 cm long with great amount of nectar stored, but is quite unpalatable (Richards 1978).

In this work we give details on an interesting correlation between morphological and physiological variation in *P. sylveirae*, showing that some queens lack typical morphological characteristics.

MATERIAL AND METHODS

Two colonies of *P. sylveirae* were collected in São José do Rio Preto – São Paulo State (20°47'07'' S 49°21'36'' W), on 31/i/2006 and 13/ii/2006, called here colony I and II respectively. The nests were collected at early evening using plastic bags with cotton balls soaked with ether. Later, the specimens were preserved in ethanol 90%.

For each colony, all queens and a sample of 50 workers were randomly chosen for measurements. The specimens were separated diagnosed according the characteristics described by Shima *et al.* (1996) and after dissected for check their status.

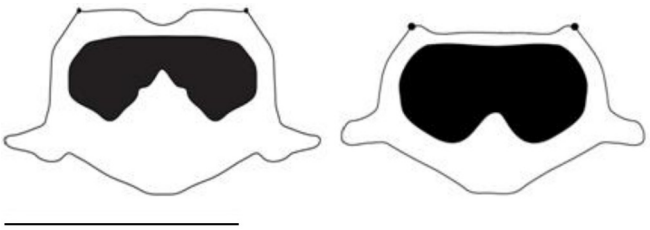

Fig. 1. Patterns of the spots in the clypeus of workers (left) and queens (right) of *Protonectarina sylveirae*. Scale bar 1.0 mm.

The following characters were measured: head width (HW); minimum interorbital distance (PML); mesoscutellar width (MSW); alitrunk length (AL); basal width tergum I (T1BH); basal width tergum I (T1BW); maximum width tergum I (T1L); partial length of forewing (WL). In order to measure the specimens, we used a set of stereomicroscope, digital camera and Axiovision 3.1 software version 5.05.10.

We used linear measures and covariance analyses in order to verify morphological differences associated with caste and size of individuals (Noll *et al.* 2004). The data was analyzed by two statistical methods: ANOVA and Discriminant Analysis (Rao 1973) in order to identify the significantly characters on caste differentiation. These analyses were performed using software STATISTICA version 5.0 (StatSoft 1995).

Wilks' Lambda values were used to infer the individual contribution of each variable to the model. The Wilks' lambda statistic for the overall discrimination is computed as the ratio of [the determinant of the within-groups variance / covariance matrix] to [the determinant of the total variance / covariance matrix]. When this value is close to 1.0, then the residual is high and the variable is not a good discriminator, while a value closer to 0 means that the residual is low and the variable is a good discriminator. In order to check the efficiency of the test, a classification matrix test was used to check the number and percent of correctly classified cases in

Table II. Wilks' λ are used to estimate the degree of contribution of each measure, individually, to the model of discriminant function. Colony II shows higher values, which indicates a smaller discrimination among castes.

Colony #	Characters	λ de Wilks
1		
	HW	0.37
	Pml	0.30
	MSW	0.29
	T_1BW	0.45
	AL	0.29
	T_1BH	0.29
	T^1L	0.29
	WL	0.29
2		
	HW	0.61
	Pml	0.52
	MSW	0.51
	T_1BW	0.59
	AL	0.51
	T^1BH	0.51
	T_1L	0.50
	WL	0.49

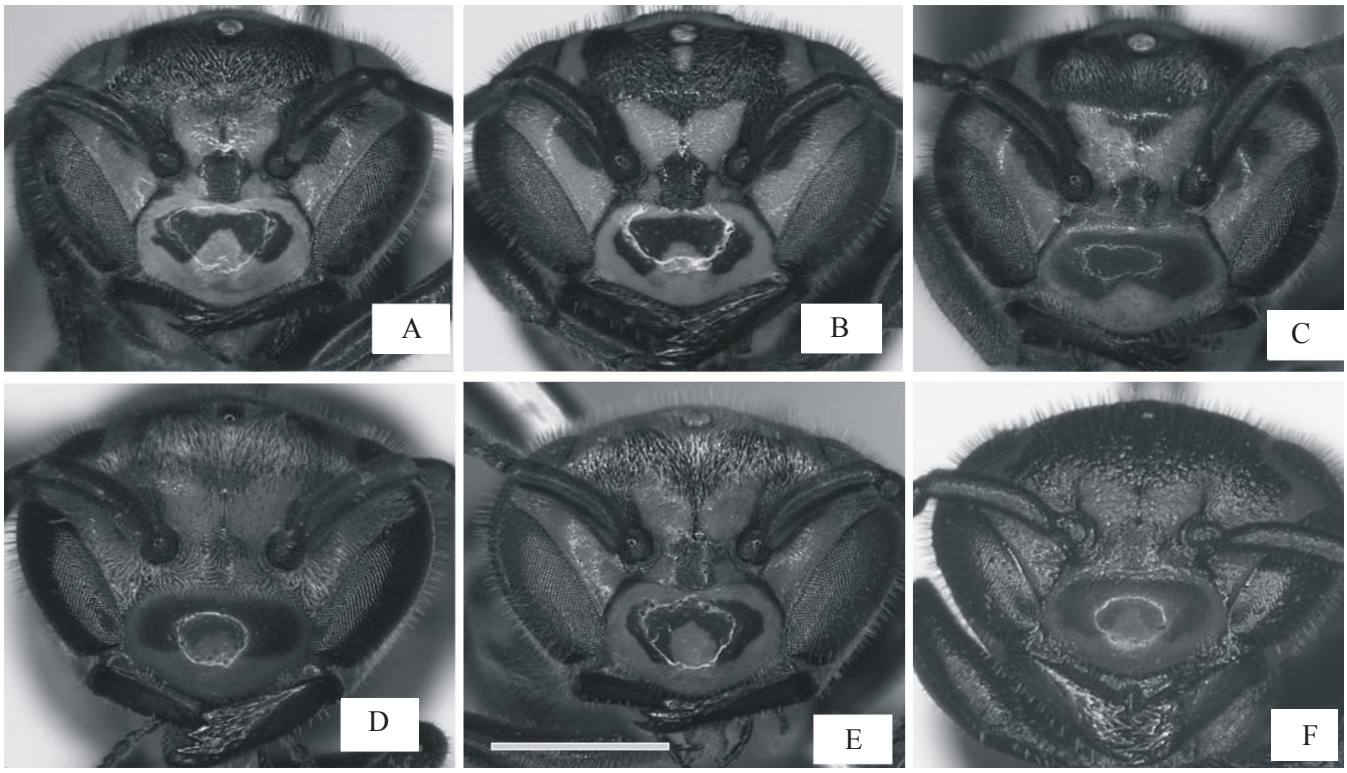

Fig. 2. Individuals of colonies I and II of *Protonectarina sylveirae*. A and B - Workers. C and D – Queens. E and F – Queens with some features of workers (Intermediates). Note the variation on clypeus spot and hairs. Scale bar 1.0 mm.

each group. This test compares the actual data with predicted results based on the discrimination model (Noll *et al*. 2004).

We also estimated the relative age of a sample of 50 workers and 100 queens from each nest, noting the progressive pigmentation of the transverse apodeme across the hidden base of the fifth sternum (Richards 1971). The color pattern of glandular development was separated in four groups as following: 1) without pigmentation (young); 2) light brown (middle-age I); 3) dark brown (middle-age II); and 4) black (old).

RESULTS

Nests: Both nests were highly populated. Nest of colony I had 43 combs and approximately 14,200 individuals, 267 of which were queens. The colony II had 54 combs and approximately 43,000 individuals, 500 of which were queens. Besides both nests had a high number of eggs, larvae and pupae, suggesting high activity of the queens in producing brood, the nest I showed more honey in their cells and more layers of meconium suggesting that this nest produced more generations (Baio *et al.* 2004; Mateus *et al.* 2004; Saito *et al.* 2004). Males were also found in both colonies, indicating a 'male-producing phase' (Noll & Zucchi 2002).

External morphology: In both colonies, with the exception of MSW from colony II, all measures showed significant differences ($p<0.01$) between queens and workers, evidencing clear caste discrimination in *P. sylveirae* (Table I). Among all measures taken, the most significant differences were HW and T_1BW.

Besides allometric differences, we noticed that workers had differences on clypeal spots and hairs shorter and sparser (Figs. 2A and B). The clypeal spots showed great shape variation and the body color was close to black). Queens had a more uniform pattern on the clypeus area, with a brownish color and denser and longer hairs all over the head (Figs. 2C and D).

We noticed variation on queens' size. The individuals, initially selected as queens by color discrimination, were classified as workers by statistical analysis, but not at all. Some females showed a brownish color in the body (supposedly queens) but with a similar color pattern found in workers (Figs. 2E and F). For these females, besides the clypeal spot, the hairs were also intermediate between queens and workers in both density and size. Beyond the features cited above, this group of females was smaller than regular queens also (Fig. 2).

Ovarian development, insemination and relative age: Three categories of ovarian development were seen in females: A) without ovarian development, from non-defined oocytes to very small, slightly defined ones; B) well developed ovaries, with well-defined and matured oocytes; and C) oocytes more developed only in the upper portion of ovary (Fig. 4). All workers dissected showed type A ovaries, and in queens we noted some variation on ovarian development. Most queens showed type B, and a few, the smaller ones with

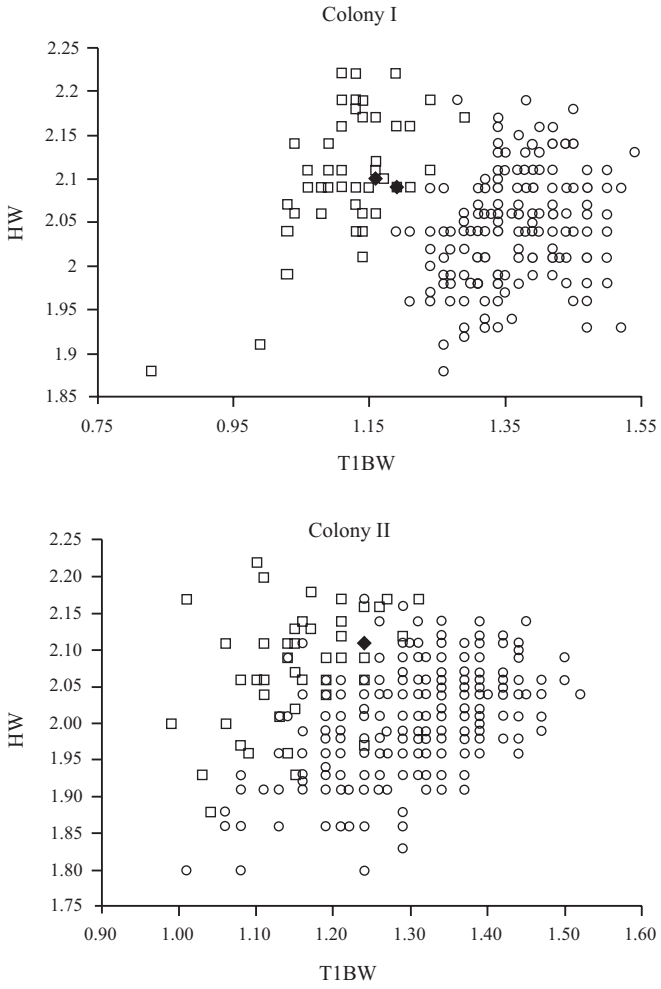

Fig. 3. Caste discrimination using characters of head (HW) and abdomen (T1BW) of colonies I and II of *Protonectarina sylveirae*. Workers (circles), Queens (squares) and Intermediates (lozenge).

lighter color and morphological intermediate pattern between regular queens and workers (see above), showed type C. We noticed that in both colonies, all queens were inseminated, excepted for those with type C.

Among the analyzed workers, we found a range from young to old females, what indicates a continuum production of workers. Among queens' sample, on the other hand, our sample had a bias towards old individuals (Fig. 5) and with fifth sternite relatively larger, in relation to workers. Younger queens were those that pointed as workers during statistically analysis. Besides, they showed ovarian development from type C.

DISCUSSION

The present work noted a clear caste differentiation in *Protonectarina sylveirae*, as previously observed by Shima *et al*. (1996, 2003) and Richards (1978).

According to Shima *et al*. (2003), *P. sylveirae* is included on this group, and the oligogynic cycle seems to be directly related with the increase on caste differentiation. Even though colony I had a smaller population density, the relative percentage of older individuals was superior (Fig. 5). Besides that, data showed that caste discrimination is larger on colony I emphasized by the smaller values of Wilks' λ, and confirms the idea that in *P. sylveirae* the differences among castes increases along colony cycle, as suggested by Shima *et al*. (2003). The differences among population densities can be resulted of the size of the swarm-founder of each colony.

Queens were different from workers, mainly in HW and T_1BW as also suggested by Jeanne & Fagen (1974) and Yamane *et al.* (1983), who reported queens with wider metasoma and smaller head. These features suggest a higher specialization among castes and social complexity in *P. sylveirae*. The increase on specialization can tend to reduce the individuals roles that each caste can develop (Gadagkar 1997). Michener (1974) suggested that the majority of queens' specialization were structural and behavioral, losses in comparison with workers and females of solitary bees.

The differences on T_1BW can reflect an intimate adaptation on queens' ovarian development, that go through changes on weight and size of metasoma, and some extents could propitiate a more adequate backing. Previously studies also showed that this measure was discriminant on *Polybia paulista*, *P. scutellaris* (White, 1841) (Noll & Zucchi 2000; Richards 1978) and *P. occidentalis* (Noll & Zucchi 2000).

Workers were responsible for a much more range of activities in comparison with queens, such as defense, nest construction and maintenance, offspring care and foraging. Females with more developed accurate environmental perceptions probably could have more success on developing these activities, favoring the development of the colony. The enlarging of the head, demonstrated by HW measure,

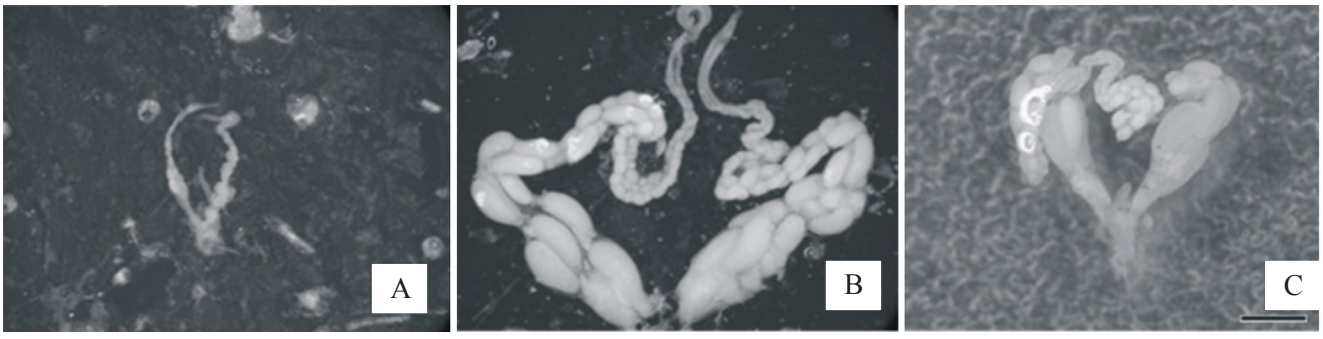

Fig. 4. Ovarian development shown by individuals of colonies I and II of *Protonectarina sylveirae*. Workers (A), Queens (B) and Intermediates (C). Scale bar 1.0 mm.

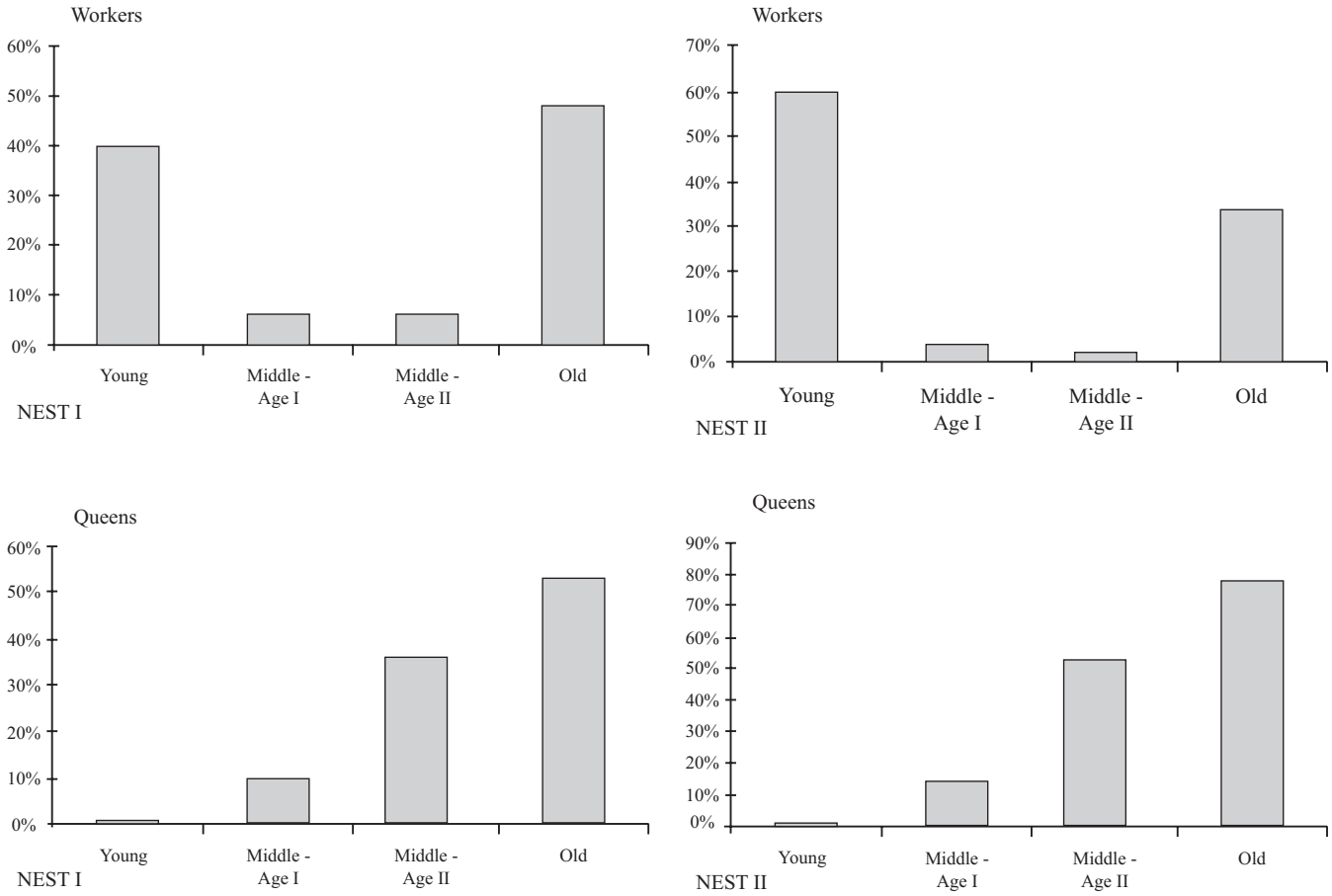

Fig. 5. Relative age from the sample of individuals from colonies I and II of *Protonectarina sylveirae.*

may suggest a development of nervous system on workers, emphasizing more specialization among castes. This phenomenon was previously seen in other Epiponini species that have large colonies (Noll *et al.* 2004).

The data show a denser pilosity on queens. As the foraging activity is restricted to workers, and the queens stay on nests most of the time, hairier queens may be more efficient to inform physical signs inside the colony and improve their perception.

The most important finding of this work was identifying smaller queens that present an intermediate condition of morphology between queens and workers. Intermediates were found in colonies studied from Shima *et al*. (2003) but not in the colonies studied by Zikán (1951) as cited by Richards (1978). The presence of intermediate shapes has been reported in many Epiponini species as cited on introduction. However, little is known about the mechanisms responsible for the caste production. Some authors suggest that intermediates are non-inseminated younger queens (West-Eberhard 1978; Gastreich *et al*. 1993; O'Donnell 1998), but other studies demonstrate divergent views, showing that in some species intermediates are similarly morphological to workers (Noll & Zucchi 2000). Richards (1971) and West-Eberhard (1978) considered that the level of ovarian development on intermediates was related with the number of queens in the colony. Our data suggest that intermediates in *P. sylveirae* have a morphological pattern more similar to queens due to their typical color and denser hairs than workers. However, statistical analysis showed that some of those females resemble workers, occupying intermediates position in the graphs showed on Fig. 3. Queens with morphological characters more similar to workers had been also reported in one of the colonies analyzed by Shima *et al*. (2003). According to the data presented in this work, this pattern may be derived of workers that can present ovaries a few more developed, caused by a better feeding, resulting in a better profit to the colony in terms of survivor (Jeanne 1991).

Unfortunately we cannot make deeper comparisons with intermediates because we found just a few of them. However it is important to note the correlation between intermediate levels of morphological characters and intermediate levels of ovarian development. Future works dealing with gene expression and caste faith should address this species.

The level of ovarian development, expressed by the difference on ovaries dimension, evidently is related with the potential of reproduction present between queens and workers. Queens probably could have a better performance if we compare the reproductive rate of these two groups. Obviously dominance interactions can also influence the difference on this potential. Ovarian size is related with body size, and larger dimensions could be important on chemical and physical signalization (Noll & Zucchi 2000). In addition, ovarian development seems to be important in queens' selection to reward the reduction on their numbers (Jeanne 1991). Intermediates showed smaller ovaries with smaller oocytes, as verified in *Pseudopolybia vespiceps* (Shima *et al*. 1998). The relative age of intermediates have no relation with the level of ovarian development, that is, apparently larger ovaries are not developed from small ones. Hence, the

intermediates may be females with smaller ovaries but with some degree of development (Shima *et al*. 1998).

West-Eberhard (1978) suggested that queens are frequently tested by workers, and their performance could be determinant in relation to their permanence in the colony. Workers could detect queen´s performance based on the offspring each queen can produce, or by the aggressive behavior of the queens as physical dominance. If this behavior applies to all epiponines, clear morphological differences found in *Protonectarina sylveirae* might be a clear sign for workers that females with all these distinctions are "good" queens, rather than "intermediate" queens with morphological similarities to workers. Maybe these intermediate females might be eliminated during the cyclical oligogyny process.

In addition to the observations of Shima *et al*. (1996, 2003), this work also showed the occurrence of intermediates with some characters of both queens and workers, these individuals, besides the fact that were non-inseminated also had intermediated measures and characteristics on hair density and clypeus shape. These findings are rarely found in Epiponini wasps, mainly in that with clear caste differentiation, as *P. sylveirae*.

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