Morphological studies on castes of *Protopolybia chartergoides* (Hymenoptera, Vespidae, Epiponini) observed in colonies during male production stage

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ABSTRACT. Morphological studies on castes of *Protopolybia chartergoides* (Hymenoptera, Vespidae, Epiponini) observed in colonies during male production stage. Social wasps are important for the study of social behavior evolution because their colonies present different degrees of caste differentiation, from morphologically similar to highly conspicuous, associated with the occasional presence of intermediate females, which bears developed ovaries but no insemination. In the Polistinae, depending on the taxon, such differentiation can be discreet or conspicuous. This work intended to study morphological and physiological differences between castes in *Protopolybia chartergoides* by using morphometric analyses associated with multivariate statistical analyses and physiological evaluations from females´ ovarian development. Results evidence low morphological and physiological differences among the castes in *P. chartergoides*, indicating three groups of females: queens, workers and intermediates. In this way, it was possible to suggest that *Protopolybia chartergoides* presents post-imaginal caste differentiation (or a very subtle form of pre-imaginal determination).

KEYWORDS. Caste differentiation; Polistinae; social wasps.

RESUMO. Estudos morfológicos sobre as castas de *Protopolybia chartergoides* (Hymenoptera, Vespidae, Epiponini) observadas em colônias durante o estágio de produção de machos. Vespas sociais são importantes para o estudo da evolução do comportamento social devido suas colônias apresentarem diferentes graus de diferenciação de castas, de morfologicamente similares às altamente conspícuas, associadas com a presença de fêmeas intermediárias, as quais desenvolvem seus ovários, mas não estando inseminadas. Nos Polistinae, tais diferenciações podem ser discretas ou conspícuas. Este trabalho teve como objetivo estudar as diferenças morfológicas e fisiológicas entre as castas de *Protopolybia chartergoides* pelo uso de análises morfométricas associada com análises estatísticas multivariadas e avaliações fisiológicas do desenvolvimento ovariano das fêmeas. Os resultados mostraram baixa diferenciação morfológica e fisiológica entre as castas de *P. chartergoides*, indicando a presença de três grupos de fêmeas: rainhas, operárias e intermediárias. Assim, foi possível sugerir que *Protopolybia chartergoides* apresenta diferenciação de casta pós-imaginal (ou uma forma muito sutil de determinação pré-imaginal).

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

Degree of morphological difference between queens and workers is an important characteristic used to infer the mechanism of caste determination and levels of reproductive conflict in insect societies. In the polistine wasps, the degree of morphological caste difference varies widely, ranging from absent to highly distinct. A lack of obvious caste differences would indicate that most females are totipotent (Strassmann *et al.* 2002, Mateus 2005), which predicts that reproductive conflict will be higher than in species with well-defined caste roles.

For Neotropical swarm-founding polistine wasps (tribe: Epiponini), West-Eberhard (1978, 1981) suggested that castes may be post-imaginally determined in some species. Indeed, female reproductives resemble non-reproductives in morphology in a number of species, and this pattern has been found in *Parachartergus* Ihering, 1904 (Mateus *et al.* 1997; Saito *et al.* 2004; Mateus *et al.* 2004), *Pseudopolybia* Dalla Torre, 1894 (Jeanne 1996; Shima *et al.* 1998), *Chartergellus* Bequaert 1938 (Mateus *et al.* 1999), *Brachygastra* Perty, 1833 (Shima *et al.* 2000), *Synoeca* Saussure, 1852 (Noda *et al.* 2003), and *Metapolybia* Richards, 1978 (Baio *et al.* 2003). Other taxa following the same pattern may be found in Noll *et al.* (2004). In some species, non-inseminated ovarian-developed females are present in colonies (termed intermediates by Richards & Richards 1951), which suggests individuals are totipotent (Strassmann *et al.* 2002). However, castes do appear to be determined pre-imaginally in *Agelaia* Lepeletier,1836 (Sakagami *et al.* 1996; Noll *et al.* 1997; Baio *et al.* 1998), *Protonectarina* Ducke,1910 (Shima *et al.* 1996b; Shima *et al.* 2003), *Polybia* Lepeletier 1836 (Shima et al. 1996a; Noll & Zucchi 2000; Noll *et al.* 2004), *Epipona* Latreille, 1802 (Hunt *et al.* 1996; Noll *et al.* 2004), and *Apoica* Lepeletier 1836 (Richards 1978) (Shima *et al.* 1994; Noll & Zucchi 2002). The presence of pre-imaginal caste determination in the basal groups of *Apoica* and *Agelaia*, suggests that this trait may be ancestral for epiponines (Jeanne 1980; Jeanne *et al.* 1995).

Protopolybia chartergoides Gribodo, 1891, occurs from NW South America (Colombia, Bolivia, Suriname, French

Guyana, Peru, Equator) to southern Brazil (Acre, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia and São Paulo) (Richards 1978). Two characteristics make this species remarkable: nests have transparent envelopes made from oral secretion (Wenzel 1998) and females are docile (Jeanne 1970), which contrasts with the common aggressiveness reputed to social wasps. Except by a few studies (Nascimento 2003), little is known about caste differences in *P. chartergoides*. According to Noll *et al.* (1996), *Protopolybia exigua* Saussure, 1854 presents slight caste differentiation between queens and workers, at least in the early stages of nest development. In a study comparing different stages of the colony cycle, Noll & Zucchi (2002) found not only slight differences but also more distinct caste differences. Some morphological differences were based not only in size but in shape, suggesting pre-imaginal determination of castes. In this work we describe the caste patterns in this species and present some possibilities for how colonies are organized.

MATERIALS AND METHODS

For this study, six colonies of *Protopolybia chartergoides* were used. They were collected in the municipal district of Nova Mutum – Mato Grosso State (S 13º 51’ 337"; W 56º 11’ 450"), in September of 2002 by Fernando B. Noll and Sidnei Mateus. Nests were collected using plastic bags with paper towel soaked in ether. All adult wasps from each colony were preserved in 96% ethanol immediately after collection. Caste was determined by examination of the ovaries and the spermatheca for insemination. Groups for analysis were defined based on ovarian development and insemination. Queens were defined as ovarian-developed inseminated females (bearing sperm in the spermatheca), intermediates were defined as unmated females with some ovarian development, and workers as unmated females without ovarian development (Noll *et al.* 2000). In this way, workers and intermediates were put together and categorized as non-inseminated, because both present were non-inseminated and intermediates were morphologically similar to workers. In order to determine insemination, the spermatheca was removed and put on a slide bearing a drop of acid fuchsine solution (1:1). The presence of sperm cells was detected under a microscope. In order to estimate the relative age of adult wasps we noted the progressive pigmentation of the transverse apodeme across the hidden base of each sternum, analyzing all individuals of each colony under a stereomicroscope. Following Richards (1971) females were classified as: without pigmentation or incipient (1), light brown (2), dark brown (3) and black (4).

For morphometric analysis females were randomly chosen in the following way: in colonies with eighty individuals or less, the whole female population was analyzed. In other colonies, eighty females were evaluated, except in colony 5, which had the largest population and 100 females were analyzed. Measurements were taken from 12 morphometric variables in each female: head width (HW); minimum interorbital distance (IDm); mesoscutellar width (MSW); alitrunk length (AL); apical height of tergum I (T1AH); length of tergum I (T1L); basal width tergum II (T2BW); partial length of forewing (WL); height of the 5th sternite (EST5V); width of the 5th sternite (EST5H); height of the 6th sternite (EST6V); width of the 5th sternite (EST6H) (Fig. 1). These measures were chosen after testing other measures seen in previous works as Shima et al. (1994) and they proved to be best possible discriminators. Measurements were taken using a stereomicroscope equipped with a digital camera linked to a computer.

For all statistical analyses we divided the females into two groups based on insemination: 1) inseminated females with ovarian development (queens), and 2) all uninseminated females (workers and intermediates). Means and standard deviations for the two groups were calculated from the five morphological measurements. One-way ANOVA was used for mean comparisons. After one-way Anova analyses, differences

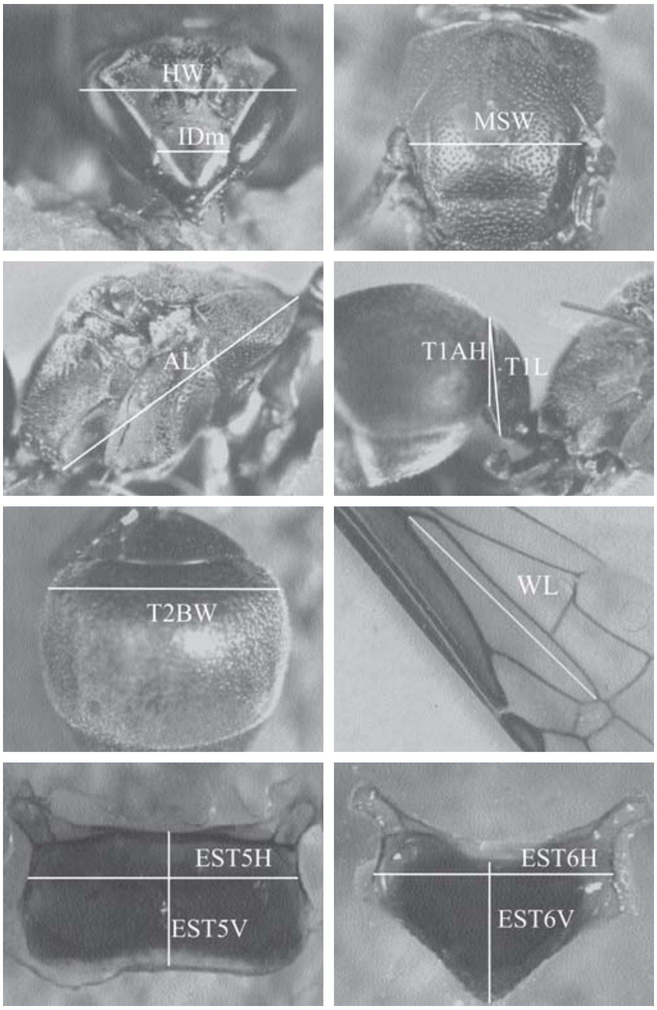

Fig. 1. Representative measures for morphometric analyses: head width (HW); minimum interorbital distance (IDm); width of mesoscutum (MSW); alitrunk length (AL); apical height of tergite I (T1AH); length of gastral tergite I (T1L); basal widths of tergite II (T2BW); partial length of the forewing (WL); width of the 5th sternite (EST5H); height of the 5th sternite (EST5V); width of the 5th sternite (EST6H) and height of the 6th sternite (EST6V).

Table I. Number of females found in analyzed the colonies of *Protopolybia chartergoides*.

Colony	Queens	Workers	Intermediates	Males	Total population	Colony characteristics
1	40	29	11	+	84	Undetermined
2	22	23	15	+	67	Nest recently settled*
3	36	19	25	+	112	Nest recently settled*
4	28	34	18	+	100	Old nest**
5	57	27	16	+	285	Old nest**
6	56	4	20	+	158	Old nest**

* with only the first brood production
** with several generations as ascertained by meconial counts.

between inseminated and non-inseminated females for each variable were tested by Bonferroni t-test (Noll & Zucchi 2002). Because multiple measures that relate to body shape are not always captured well in single comparisons, stepwise discriminant function analysis was used to see if combinations of variables could be useful in predicting caste (Rao 1973). In this method, variables are successively added to the model based on the higher *F to enter* values, adding no more variables when the F-ratio is no longer significant. However, variables that enter the model early may be completely subsumed by separate variables that enter later. The significance of Wilks' lambda (an estimate of common variance between sets of varieties) was used to determine the degree to which separate measures contributed to the final model. This is an alternative to the using an *F to remove* at each step. Variables that appear in the final model but do not have significant F ratios represent variance components that are explained by some combination of the other variables also in the model, and therefore no longer contribute to the discrimination itself. Wilks' lambda varies from zero to one, the lower the value, and the greater the significance. 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 each group (Noll & Zucchi 2002). A canonical analysis was also employed in order to determine if the possible variation was due to size differences or due to differences in body proportions (Noll *et al.* 2004).

RESULTS

Ovarian development and age. We classified ovarian development according to three categories, type A, B, and C. Type A ovaries possessed non-defined oocytes through very small, slightly defined oocytes. Type B ovaries comprised small oocytes and oocytes reduced in length through oocytes larger in length. Type C ovaries had oocytes ranging from completely developed to reduced in length, with a pair of mature oocytes

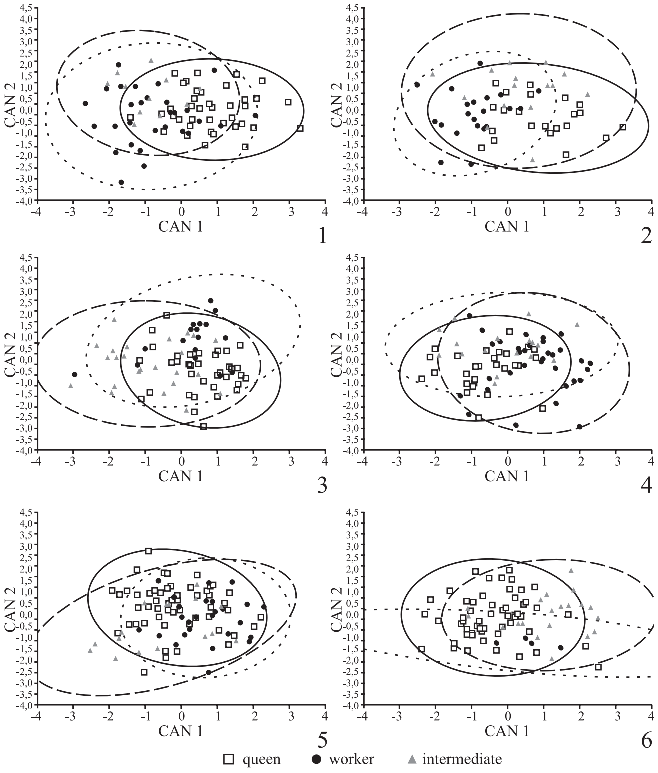

Fig. 2. Caste discrimination in the analyzed colonies of *Protopolybia chartergoides* based on the first two canonical roots. Each ellipse encompasses 95% of the variation.

Table II. Age of queens, workers and intermediates based on the color of the 5th gastral sternite in *Protopolybia chartergoides*. 1- without pigmentation (youngest females); 2 – light gray; 3 – dark gray; 4 – black (oldest females).

Colony	Relative Age (%) Caste	1	2	3	4
1	Queen	41	28	18	13
	Worker	3	31	35	31
	Intermediate	0	34	33	33
2	Queen	5	19	71	5
	Worker	0	14	45	41
	Intermediate	7	14	57	22
3	Queen	11	36	44	9
	Worker	16	37	37	10
	Intermediate	26	35	30	9
4	Queen	0	22	37	41
	Worker	0	9	42	49
	Intermediate	0	0	56	44
5	Queen	0	9	50	41
	Worker	0	4	44	52
	Intermediate	0	6	69	25
6	Queen	0	3	52	45
	Worker	0	0	25	75
	Intermediate	0	0	61	39

Table III. Means, standard deviations and F values of ANOVA for the analyzed colonies of *Protopolybia chartergoides*.

Characters	Colony 1			Colony 2			Colony 3		
	Inseminated (N=40)	Non-inseminated (N=40)	Bonferroni T-test	Inseminated (N=22)	Non-inseminated (N=38)	Bonferroni T-test	Inseminated (N=36)	Non-inseminated (N=44)	Bonferroni T-test
Head									
HW	2.33±0.04	2.32±0.05	#	2.32±0.03	2.32±0.03	#	2.31±0.03	2.28±0.04	**
IDm	0.78±0.03	0.76±0.03	***	0.74±0.04	0.76±0.03	**	0.77±0.03	0.77±0.03	#
Mesosoma									
MSW	1.80±0.06	1.82±0.09	#	1.85±0.05	1.88±0.06	#	1.85±0.03	1.81±0.07	#
AL	3.28±0.09	3.28±0.12	#	3.31±0.11	3.31±0.07	#	3.28±0.08	3.24±0.11	#
Metasoma									
T1L	0.93± 0.1	0.86±0.13	**	0.88± 0.12	0.87±0.12	#	0.88±0.11	0.88± 0.13	#
T1AH	1.30±0.07	1.30±0.07	#	1.28±0.07	1.28±0.05	#	1.28±0.05	1.26±0.07	#
T2BW	2.41±0.11	2.37±0.14	#	2.35±0.11	2.39±0.12	#	2.40±0.11	2.41±0.10	#
EST5V	1.14±0.03	1.13±0.04	#	1.11±0.03	1.13±0.03	#	1.13±0.04	1.10±0.04	**
EST5H	2.81±0.09	2.75±0.07	*	2.66±0.09	2.71±0.07	**	2.61±0.11	2.59±0.07	#
EST6V	1.37±0.08	1.33±0.04	**	1.45±0.06	1.48±0.06	#	1.32±0.04	1.31±0.04	#
EST6H	2.10±0.08	2.07±0.08	**	2.08±0.07	2.11±0.07	#	2.03±0.11	2.00±0.07	**
Wing									
WL	2.68±0.09	2.70±0.08	#	2.75±0.04	2.80±0.05	*	2.78±0.06	2.73±0.07	#

*$P < 0.001$, **$P < 0.02$, ***$P < 0.05$, # No significant ($P > 0.05$)

Characters	Colony 4			Colony 5			Colony 6		
	Inseminated (N=28)	Non-inseminated (N=52)	Bonferroni T-test	Inseminated (N=57)	Non-inseminated (N=43)	Bonferroni T-test	Inseminated (N=56)	Non-inseminated (N=24)	Bonferroni T-test
Head									
HW	2.43±0.19	2.39±0.17	#	3.40±0.24	3.42±0.19	#	3.40±0.24	3.42±0.19	#
IDm	0.79±0.071	0.79±0.06	#	1.18±0.09	1.21±0.08	#	1.18±0.09	1.21±0.08	#
Mesosoma									
MSW	1.95±0.17	1.93±0.14	#	2.67±0.23	2.68±0.21	#	2.67±0.23	2.68±0.21	**
AL	3.44±0.22	3.37±0.23	#	4.80±0.38	4.79±0.31	#	4.80±0.38	4.79±0.31	**
Metasoma									
T1L	1.00± 0.14	0.93±0.13	***	1.29±0.27	1.32±0.27	#	1.29±0.27	1.32±0.27	**
T1AH	1.34±0.071	1.35±0.10	#	1.94±0.21	1.97±0.17	#	1.94±0.21	1.97±0.17	#
T2BW	2.47±0.24	2.42±0.21	#	3.70±0.29	3.76±0.24	#	3.70±0.29	3.76±0.24	#
EST5V	1.12±0.020	1.12±0.03	#	1.10±0.06	1.10±0.07	#	1.10±0.06	1.10±0.07	#
EST5H	2.67±0.076	2.65±0.09	#	2.56±0.11	2.57±0.15	#	2.56±0.11	2.57±0.15	#
EST6V	1.31±0.033	1.29±0.05	#	1.27±0.06	1.29±0.06	#	1.27±0.06	1.29±0.06	#
EST6H	2.06±0.057	2.06±0.12	#	1.97±0.11	1.98±0.14	#	1.97±0.11	1.98±0.14	#
Wing									
WL	2.86±0.22	2.82±0.18	#	3.96±0.29	3.99±0.26	#	3.96±0.29	3.99±0.26	#

*$P < 0.001$, **$P < 0.02$, ***$P < 0.05$, # No significant ($P > 0.05$)

plus six or more less developed oocytes at the base of the ovary (see Noll *et al.* [2000] for a diagram of these ovary types). Females classified as workers possessed ovaries of type A, as intermediates, type B, and as queens, type C. Insemination was found only in females with ovaries of type C. These patterns are commonly found in several other epiponines, and represent a continuum. There were a large number of queens in all colonies (Table I). In general, adult females in all colonies were composed mostly by older individuals (age classes 3 and 4), except in colony 1, where younger queens were found (69.2% of age classes 1 or 2; Table II). Among the three types of adult females, intermediates were the group that comprised a higher rate of younger individuals.

Morphometrics. ANOVA showed that except for colony 5 (Table III), all colonies presented at least one significant measured character separating inseminated (queens) and uninseminated females (workers and intermediates). In colonies 1, 3, 4 and 6 inseminated females were larger than uninseminated females in at least one character (Table III). In colony 2 inseminated females were smaller in three characters (Table III). Bonferroni t-tests showed some differences between inseminated and non-inseminated females, but based in a few measures. In colonies 2, 3, and 6, three of twelve characters were statistically different (Table III), in colony 1, five characters were statistically different (Table III), in colony 4 just one character was statistically different and in colony 5 no characters were statistically different (Table III).

In the evaluation of overall size variation, discriminant analyses showed a low differentiation pattern for all colonies (Table IV). High values of Wilks' Lambda (above 0.5; Table IV) indicate that no single character could discriminate castes, but only a combination of them. In general, the most discriminant characters for most colonies were alitrunk length (AL), and minimum interorbital distance (IDm). Group comparisons (Table V) after discriminant analyses showed that both inseminated and uninseminated females were only partially classified correctly, indicating a high overlap between these two groups in morphological characters. Moreover, the canonical analysis (Fig. 2) also shows such an overlap. Only two discriminant vectors were obtained and most variation was based on the first root, which, by definition, indicates differences are due to size variation. Because no variation between castes was found in the second root, it is clear that only size, and not shape differences, may separate castes in *P. chartergoides*.

DISCUSSION

Our data show slight caste differences in both morphological and physiological aspects, as previously observed (Nascimento 2003). Low differentiation between castes is a widespread trait in the Epiponini, characterizing the genera *Protopolybia*, *Parachartergus*, *Pseudopolybia*, *Polybia*, *Angiopolybia* Lepeletier, 1836, *Chartergellus* and *Brachygastra* (Mateus *et al.* 2004; Noll *et al.* 2004). In colonies 1, 2, 3, 4, and 6 at least one measure was statistically different between inseminated and non-inseminated females (Table III), while in colony 5 no significant differences were found. Because some colonies had more significantly different morphological characters than in others (Table III), we suggest that castes vary from slight to moderate differences. Based on discriminant analyses, small differences were found in all colonies. High Wilks' Lambda values (Table IV) explain this result, since, for some colonies, morphological variables were able to discriminate castes only when associated, and not when considered independently.

Epiponine wasps alternate between polygyny and monogyny through the colony cycle in a process called cyclical oligogyny (West-Eberhard 1978). During a process of queen reduction, queens are challenged by the workers (West-Eberhard 1978, 1981; Herman *et al.* 2000) and they may be eliminated if they behave submissively. If such queen-removal is related to morphological characters, such as queen size, caste differences can change throughout the colony cycle (Noll & Zucchi 2002). In *P. chartergoides*, even with low discrimination, group comparisons (Table V) of inseminated and uninseminated individuals were classified correctly at a high rate in some colonies, especially colony 6 which contained the oldest queens. This is consistent with the hypothesis that during the process of cyclical oligogyny in *P. chatergoides* the process of queen elimination is based on size as suggested by Noll & Zucchi (2002).

Table IV. Discrimination between inseminated and non-inseminated females in all analyzed colonies of *Protopolybia chartergoides* after a forward stepwise discriminant function analyses.

Colony	Character	Wilks' Lambda	F	*p*
1	EST5H	0.94	4.21	0.04
	IDm	0.90	7.82	*
	T1L	0.92	6.17	0.02
	EST6V	0.91	6.75	0.01
	MSW	0.93	5.25	0.03
	HW	0.93	4.83	0.03
	WL	0.95	3.44	0.07
	EST5V	0.96	2.64	0.11
	T1AH	0.96	2.56	0.11
	T2BW	0.98	1.32	0.26
2	WL	0.84	10.5	*
	IDm	0.75	3.19	0.08
	AL	0.74	2.57	0.11
	EST5H	0.73	2.10	0.15
	HW	0.73	1.71	0.20
3	HW	0.83	7.18	*
	EST6H	0.82	5.94	0.02
	IDm	0.80	4.16	0.05
	EST5H	0.80	4.29	0.04
	T2BW	0.78	2.98	0.09
	EST5V	0.78	2.03	0.16
4	T1L	0.91	8.75	*
	T1AH	0.88	6.45	0.01
	EST5V	0.84	2.47	0.12
	AL	0.85	3.46	0.07
	IDm	0.83	1.31	0.26
5	IDm	0.93	1.97	0.16
	AL	0.97	6.48	0.01
	EST6V	0.93	2.70	0.10
	T2BW	0.93	2.19	0.14
6	T1L	0.75	3.31	0.07
	MSW	0.74	2.11	0.15
	AL	0.76	3.82	0.05
	IDm	0.74	1.89	0.17
	HW	0.74	1.62	0.21
	EST6H	0.75	3.22	0.08
	EST5H	0.75	2.74	0.10

*$P<0,01$

We observed a high level of ovarian development in non-inseminated females (Table 1). Intermediates are widespread among epiponines, usually when caste differentiation is low (Shima *et al.* 1998; Mateus *et al.* 2004). As seen in *Protopolybia exigua* (Simões 1977; Noll *et al.* 1996), intermediates in *P. chartergoides* may represent a phase in the life of a typical worker. This suggests that adult females are totipotent in this species (Strassmann *et al.* 2002; Noll *et al.* 2004; Mateus *et al.* 2004, Mateus 2005), because some young workers, if inseminated, may be able to start a new nest as queens (they may later be eliminated) (West-Eberhard 1978; Noll & Zucchi 2002).

Colony size in *P. chartergoides* is quite small, reaching only a few hundred (maximum of 285 females in our study,

Table V. Classification matrix for inseminated and non-inseminated females after discriminant function analyses in the colonies of *Protopolybia chartergoides*.

Colony			Predicted classification		Corrected classified females (%)
			Inseminated	Non-Inseminated	
1	Inseminated	Observed classification	31	9	77.5
	Non-Inseminated		7	33	82.5
2	Inseminated		12	10	54.6
	Non-Inseminated		3	35	92.1
3	Inseminated		26	10	72.2
	Non-Inseminated		10	34	77.3
4	Inseminated		12	16	42.9
	Non-Inseminated		7	45	86.5
5	Inseminated		45	12	79
	Non-Inseminated		23	20	47.5
6	Inseminated		52	4	92.9
	Non-Inseminated		12	12	50

Table I). We could estimate the maximum number of females because colonies 2 and 3 were in the first brood generation, and colonies 4, 5 and 6 were old colonies (personal observation). Several epiponines have very large colonies (Noll *et al.* 2004), reaching up to millions (Zucchi *et al.* 1995). As suggested by Bourke (1999), as the colony size increases, workers experience a decrease in their chances of becoming reproductive substitutes, which favors increased mutual reproductive inhibition (worker policing, Ratnieks 1988). As the workers' reproductive potential decreases, the level of reproductive dimorphism between castes increases (Wilson 1971; Michener 1974; Oster & Wilson 1978; Hölldobler & Wilson 1990; Alexander *et al.* 1991; Wheeler 1991; Bourke 1999). This morphological skew (Bourke 1999) helps to explain why societies composed of a few individuals have small differences between castes, and those with many individuals have a more pronounced distinction. For these reasons, small societies should be characterized by direct reproductive conflict. In contrast, conflicts in larger societies should be predominantly on brood composition, i.e. the production of males or females, and the members of these societies should be relatively more "resigned" with the manipulation of their castes (Bourke 1999). Because *P. chartergoides* possessed small colonies, a large proportion of queens in all colonies and many intermediates, we suggest that predictions for species with low morphological skew should apply to this species. Intermediates are also common in other *Protopolybia* species (Naumann 1970, Simões 1977, Noll *et al.* 1996, Noll & Zucchi 2002). Considering the presence of a large number of queens in all colonies, it is possible that queen-worker conflict might be higher than in other epiponines, and that the intermediates in colonies may be continuous male producers.

In conclusion, because we found low morphological and physiological differentiation, we suggest that castes in *P. chartergoides* are determined post-imaginally (Mateus *et al.* 2004), but size may play an important role during the process of queen elimination during cyclical oligogyny (Noll & Zucchi 2002).

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