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Research article

Use of artificial arenas to predict the social organisation of halictine bees: Data for fourteen species from Chile

L. Packer

Department of Biology, York University, 4700 Keele St., Toronto, Ontario, M3J 1P3 Canada, e-mail: bugsrus@yorku.ca

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Abstract. I studied the behavioural profiles of fourteen species of Chilean halictine bee using the circle tube apparatus. Interactions were classified as aggressive, avoidance or cooperative. One species, *Corynura chloris,* is believed to be semisocial and its behaviour was largely aggressive, *Ruizantheda mutabilis* is known to be communal and its behaviour was predominantly cooperative whereas *Penapis toroi* is a member of a subfamily from which only solitary behaviour is known and it primarily exhibited avoidance behaviours. As a result, I suggest that the relative frequency of these different behaviours may be useful in establishing the social organisation of species. Based upon comparisons of behavioural profiles, I predict the social organizations of the remaining species and suggest that at least some of the bees in the populations of *Ruizantheda proxima*, *Caenohalictus dolator*, *Ca. species A* and *Pseudagapostemon pississi* investigated exhibit communal behaviour; those of *Co. patagonica* and *Co. herbsti* likely have a reproductive division of labour, probably within semisocial societies, and that the populations of *Co. corinogaster, Co. melanocladius, Ca. pygosinuatum, Ca. cuprellus* and *Lasioglossum aricense* studied are probably solitary. Phylogenies suggest that solitary behaviour in *Ca. pygosinuatum* and *Ca. cuprellus* is reversed from communal behaviour and solitary behaviour in *L. aricense* may be a reversal from eusociality. The response "back and follow" is not indicative of dominance or cooperation as it correlated only with the frequency of avoidance interactions among the taxa studied. I discuss the utility of the circle tube apparatus in the identification of populations worthy of more detailed sociobiological investigation.

Keywords: Halictidae, social behaviour, communal, solitary, reproductive division of labour.

Introduction

Halictine bees are well known for their diversity, both in number of species and variety of social behaviours (Michener, 1990, 2000; Packer 1993; Wcislo and Danforth, 1997; Yanega, 1997). Many have been shown to possess either eusocial, semisocial or communal behaviours (sensu Michener, 1974; reviewed by Yanega, 1997) within their repertoires. But, unlike most other higher-level hymenopteran taxa containing social species (such as the Formicidae, Vespinae and Apini), many are solitary while others are socially polymorphic (Packer, 1997; Wcislo and Danforth, 1997; Soucy, 2001; Richards et al., 2003). This behavioural variation has been placed within a phylogenetic context in numerous studies (Packer, 1997, 2000; Danforth et al., 2003, 2004; Danforth, 2002; Janjic and Packer, 2002; Soucy and Danforth, 2002). Nonetheless, approximately half of the genera and the vast majority of species (over 2400) are unknown behaviourally. With interpopulation social behavioural variation pronounced in most of the species that have received multiple studies (e.g. Sakagami and Munakata, 1972; Eickwort et al., 1996; Packer, 1990; Plateaux-Quénu et al., 2000; Soucy and Danforth, 2002; Wyman and Richards, 2003) it is abundantly clear that most behaviourally interesting units in these bees will never be studied in detail. Nonetheless, given the apparent phylogenetic lability of social organization in the subfamily, it is important to obtain at least rudimentary behavioural data for as many populations of as many species as possible. However, given the sheer number of genera, species and populations requiring study and the difficulties associated with finding nest sites for many of even the more common ones, it is clear that a short-cut approach is required if we are to obtain a better understanding of the pattern of social evolution of this group across broad phylogenetic and biogeographic ranges.

The circle tube apparatus (Breed et al., 1978) has been used to study a number of halictines and also some members of the family Andrenidae (Paxton et al., 1999; Grixti et al., 2004). Previous applications have compared behavioural profiles among different caste pairs (Breed et al., 1978; Pabalan et al., 2000), among different species (McConnell-Garner and Kukuk, 1997; Wcislo, 1997a) or among populations of a single species (Kukuk and Decelles, 1986) including one that is socially polymorphic (Soucy, 2001). The behavioural profiles of communal bees are substantially different from those that are solitary or have a reproductive division of labour, exhibiting more tolerance (Kukuk, 1992), and even trophallaxis among unrelated, unfamiliar individuals (Kukuk and Crozier, 1990). In contrast, species known to exhibit a reproductive division of labour tend to interact more aggressively (Packer et al., 2003) and solitary ones with both less aggression and less cooperation (McConnell-Garner and Kukuk, 1997; Packer, 2005). These results suggest the possibility that the circle tube apparatus might be useful in suggesting the social organization of populations which have not been studied behaviourally.

In this paper, I report the results of studies of some Chilean halictine bees using the circle tube apparatus. Some of the species have previously been studied at their nest sites and been shown to be social (Claude-Joseph, 1926) whereas others are behaviourally completely unknown. One of the objectives of this paper is to establish whether the behavioural profiles of bees interacting in circle tubes fall into clusters that may imply particular socialities.

Chile is a long, narrow country (Fig. 1) with extreme variation in climate. In the mountains of the extreme north, where *Ca. dolator* (Vachal), *Ca. species A* and *Ca. cuprellus* (Vachal) were studied (Table 1), unpredictable summer rains (Betancourt et al., 2000) are the main stimulus for flowering and hence bee activity. In the coastal northern regions, moisture comes from sea spray, fog (camanchaca) and urban and agricultural sources. This can result in an extended period of flowering, such as seems to be the case where *L. aricense* (Schrottky) was studied. In the southern portions of the Atacama desert, where *Penapis toroi* Rozen*, Ca. pygosinuatum* Rojas and Toro, and some *Pseudagapostemon pississi* (Vachal) were observed, and in the central valley, where I studied *Corynura herbsti* (Alfken) and *Ruizantheda mutabilis* (Spinola), the winter rains are somewhat more predictable than the summer rains further north and occasional rainfall at other times of year extends the period of bee activity. Further south, in the valdivian forest region, where *Co. chloris* (Spinola)*, Co. patagonica* (Cockerell)*, Co. melanocladius* (Cockerell)*, Co. corinogaster* (Spinola)*, R. proxima* (Spinola) and the remaining *Ps. pississi* were investigated, the winters are wet and cool and the summers not very long but with considerable and generally reliable rainfall giving a comparatively abundant, but somewhat short time span of flowering.

Some of the data included here on two species, *Penapis toroi* and *Corynura chloris,* were presented previously as part of an assessment of the effect of marking upon behaviour (Packer, 2005).

Figure 1. Map of Chile showing location of study areas. 1. Zapahuira, 2. Azapa, 3. Chañaral, 4. Llanos de Challe, 5. Fray Jorge, 6. Colliguay, 7. Angol, 8. Valdivia, 9. Aguas Calientes. See Table 1 for species studied at each locality.

Methods

Bee samples

Table 1 shows the species studied, the dates, localities and sample sizes obtained and the dimensions of the plastic tubing used. *Corynura chloris* is a widespread species believed to have at least some semisocial colonies in central Chile (Packer, 2005). The information presented in Claude-Joseph (1926) indicates clearly that *R. mutabilis* has communal colonies whereas *Co. herbsti* has a few females per nest, although whether as a

Table 1. List of species studied, localities, dates, sample sizes and tubing diameter for 14 species of Chilean Halictine Bee studied using the circle tube apparatus.

Species	Region / Locality	Date Month. Year	Number of pairs studied	Tubing inner diameter (mm)
P. toroi	III / Chañaral	X. 2000	8	6
R. mutabilis	Metropolitano / Colliguay	IV. 2001	16	6
Co . chloris	X / Valdivia	X. 2000	8	6
Ca. dolator	I / Zapahuira	IV. 2001	10	6
Ca. cuprellus	I / Zapahuira	IV. 2001	6	5
Ca. pygosinuatum	IV / Fray Jorge	X. 2001	10	6
Ca. species A	I / Zapahuira	IV. 2001	9	6
Ps. pississi	III / Llanos de Challe & VIII / Angol		8	6
R. proxima	X / Valdivia	X. 2000	9	7
L. aricense	I / Azapa	IV. 2000	10	5
Co. corinogaster	X / Valdivia	X. 2000	9	6
Co. herbsti	Metropolitano / Colliguay	X. 2000	3	6
Co. patagonica	X / Aguas Calientes	X. 2000	10	6
Co. melanocladius	X / Valdivia	X. 2000	5	6

communal society or with a reproductive division of labour remains uncertain. Similarly unclear is his account of *R. proxima* (as *Halictus rubella* Halliday), which suggests, that nests might be inhabited by one or two females. No behavioural information is available for any of the other species included here. However, the tribe Rophitinae, to which *Penapis toroi* belongs, is not known to contain social species and an earlier study aimed at examining the effects of marking upon circle tube behaviours provided data consistent with this species being solitary (Packer, 2005).

Voucher specimens of all species are housed in the Packer bee collection at York University.

Experimental methods

I collected bees from flowers and retained them in 1.5 ml eppendorf tubes until use at most 30 min later to avoid physiological deterioration that may accompany capture (Pabalan et al., 2000). I placed the two interactants into the circle tube from opposite ends simultaneously as dominance behaviours can be more common in the bee placed within the tube first (Wcislo, 1997a). Observations lasted for half an hour and the plastic tubing was discarded after a single use. Individuals were used in only one circle tube experiment and were killed afterwards by preservation in buffered formalin (Humason, 1973) for later measurement and dissection using standard techniques (Abrams and Eickwort, 1980). Individuals of *Ca. pygosinuatum* could not be dissected due to storage malfunction. Unmarked bees were used in the experiments as it was previously shown that marking resulted in changes in the relative frequencies of behaviours such that differences between samples may be obscured (Packer, 2005). Consequently, individual interactants could not be differentiated, but this does not influence the conclusions drawn.

Behavioural repertoires

In previous studies (summarized by Packer, 2005), only behaviours in which one or both bees approached head on and came within a defined distance from one another, frontal encounters, have been analysed. This approach is retained here. A frontal encounter is defined as the bees coming within one body length of each other, head to head. Behaviours were classified as aggressive, cooperative or avoidance based upon previous studies (e.g. Bell and Hawkins, 1974; Breed et al., 1978; Kukuk, 1992); which should be consulted for definitions and descriptions of the behavioural interactions.

"Back and follow" where one bee backs away, while the other follows it occurs in the nest of at least some eusocial species when one individual leads another to a brood cell or some other part of the nest (Breed and Gamboa, 1977; Buckle, 1984; Pabalan et al., 2000). This behaviour has been taken to indicate tolerance of social interaction or cooperation (Wcislo, 1997a) and, because it is generally the queen that does the leading, it has also been used to determine which individual is dominant (Pabalan et al., 2000). In the taxa investigated here, this behaviour seemed less ritualized or deliberate than it does in *Halictus ligatus* (Pabalan et al., 2000; Packer, unpubl. obs.). Whether it represents cooperative, dominance or avoidance behaviour is something that requires additional study and this is investigated. Given this uncertainty, "back and follow" was left out of the calculations of the proportion of interactions that were aggressive, cooperative or avoidance.

Scoring of frontal encounters

I only scored the outcome of an interaction as avoidance if this was the initial and only response to the approach even though aggressively or cooperatively interacting bees must eventually move away from each other before another frontal encounter can occur. Note that the semantic difficulty of considering an outcome of an interaction as avoidance of an interaction can be readily side-stepped by considering avoidance to represent avoidance of further interaction (something which the structure of the circle tube apparatus renders temporary unless both bees remain stationary). I coded each frontal encounter for only one behavioural outcome except when cooperative behaviours were accompanied by aggressive acts. For example, when one member of a pair makes a C-posture just prior to permitting the other to pass. In these instances I considered the two interactions separately.

The population level responses presented were obtained by weighting each pair of interactant equally. Thus, irrespective of the number of interactions a particular pair had, each pair contributed equally to the overall proportions of the three main categories of behaviour noted above.

Statistical analysis

Because of large differences in the activity levels of pairs of bees both within and among species, it is standard for circle tube behaviours to be analysed using the relative frequencies of behaviours per encounter.

Such data are ratios the statistical properties of which are unknown (for a discussion of this problem with circle tube data see Wcislo, 1997a).

I plot the relative frequency of cooperative behaviours exhibited by a pair (or averaged across pairs within a species) against the frequency of aggressive interactions as this is an easy way to visualize differences among pairs (or more inclusive units such as populations) (Packer et al., 2003). Various techniques were tried to increase the discrimination among pairs or populations but none clearly improved upon the simpler approach adopted here.

Whether the behaviour "back and follow" seems indicative of dominance, cooperative or avoidance behaviour was tested with rank correlation across species.

Results

Interspecific comparisons of species with surmised social organisation

Table 2A shows summary statistics for behavioural profiles for the three samples whose social organisation is considered to be known or at least well predicted. The results for individual pairs are shown in Figure 2. The results are easy to interpret. The communal bee, *R. mutabilis*, exhibited very high levels of cooperative behaviour, 85.3 % of all interactions, and low levels of aggression, 1.9 %. In contrast, the species suspected of having a reproductive division of labour, *Co. chloris,* had a high frequency of aggressive interactions (56.3 %) and a low level of cooperation (8.2 %). Lastly, the solitary *Penapis* had low levels of both aggression and cooperative behaviours (10.4 and 13.3 % respectively), but high levels of avoidance (76.2 %). Note that there are slight differences in the statistics presented for the last two species from those in Packer (2005) because the data here are from a longer observation period.

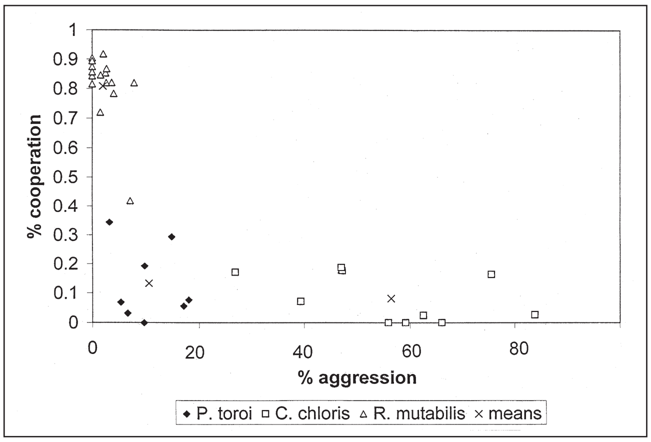

Figure 2. Proportion of all interactions that were cooperative versus those that were aggressive for the all pairs of the three samples considered to be of known social organization.

Behaviours of species with unknown social organisation

Table 2B shows the relative frequencies of cooperative, avoidance and aggressive interactions for 11 species whose social organisation is unknown, although several are either known to have, or suspected of having, multi-female colo-

Table 2. Behavioural statistics for 14 species of Chilean halictines studied in the circle tube apparatus, sample sizes are given in Table 1.

Species	aggression	avoidance	cooperation	pass success	mutual avoidance	C-posture	back & follow
A. With social data							
P. toroi	10.4	76.2	13.3	61.6	26.3	11.1	6.4
R. mutabilis	1.9	17.2	80.9	98.2	15.8	0.0	0.2
Co . chloris	56.3	35.5	8.2	56.9	21.8	53.8	2.9
B. Without social data							
Ca. dolator	2.4	16.2	81.5	97.2	16.4	0.0	0.0
Ca. cuprellus	20.7	60.1	19.1	62.2	12.9	41.1	6.8
Ca. pygosinuatum	28.2	34.9	37.0	88.0	11.8	17.4	0.0
Ca. species A	3.9	25.8	70.3	97.2	21.5	8.3	3.3
Ps. pississi	12.6	35.9	51.5	82.8	19.9	50.0	2.8
R. proxima	5.6	31.6	63.1	93.6	4.2	6.3	0.0
L. aricense	25.1	50.6	24.3	87.8	40.3	35.9	1.6
Co. corinogaster	28.5	55.3	16.1	77.4	13.1	51.1	0.9
Co. herbsti	40.3	55.4	4.2	62.5	21.3	59.1	2.3
Co. patagonica	40.4	48.8	13.9	75.9	19.7	25.3	1.4
Co. melanocladius	26.8	48.4	24.6	80.9	32.2	7.1	1.0

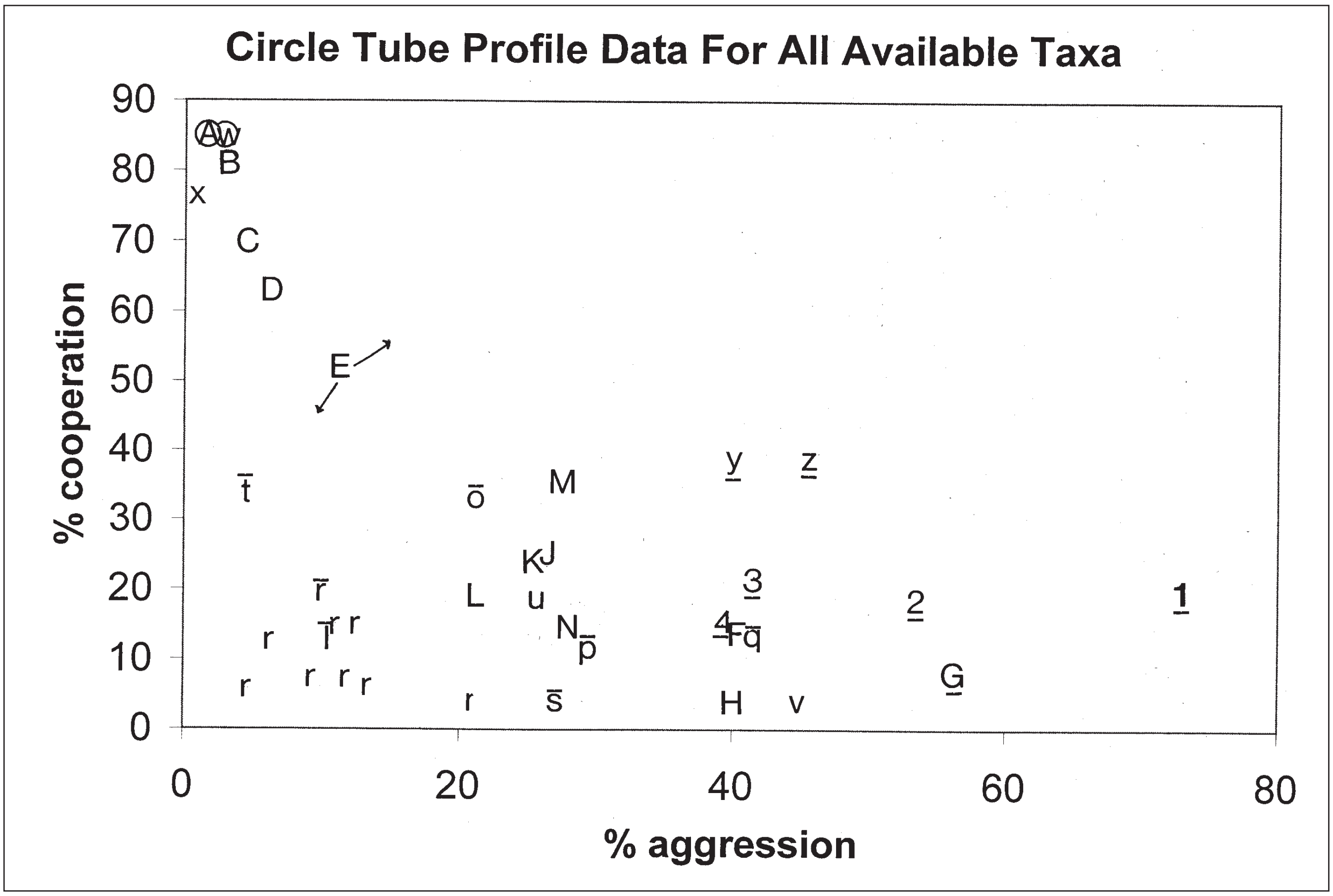

Figure 3. Sample means for the proportion of cooperative versus proportion of aggressive interactions. Data represented by capital letters refer to data presented herein, lower case and numbers refer to data from the literature, with citation, or Packer, (unpublished data), where no citation is provided. Species believed to exhibit a reproductive division of labour (through studies in addition to circle tube investigations) are underlined, those believed to be communal are circled, and those believed to be solitary are overlined. Species and references to circle tube experiments and/or social organisation are as follows: A. *R. mutabilis* (Claude-Joseph, 1926) B. *Ca. dolator*, C. *Ca.* sp. A, D. *R. proxima*, E. *Ps. pississi*, F. *Co. patagonica*, G. *Co. chloris* (Claude-Joseph, 1926), H. *Co. herbsti*, I. *P. toroi,* J. *Co. melanocladius*, K. *L. aricense*, L. *Ca. cuprellus*, M. *Ca. pygosinuatum*, N. *Co. corinogaster*, o. *L. (Ctenonomia)* sp. (McConnel-Garner and Kukuk, 1997), p. *L. platycephalum* (Rayment) (McConnell-Garner and Kukuk, 1997), q. *L. figueresi* Wcislo (Wcislo, 1997), r. various rophitines (e.g. Eickwort et al., 1986), s. *Thrincohalictus prognathus* (Perez) (Packer, 2000), t. *Dieunomia nevadensis* Cresson (Kerfoot, 1964), u. *Nomia fedorensis* Cockerell. v. *Dieunomia mesillae* Cockerell. w. *L. hemichalceum* (Cockerell) (Kukuk, 1992), x. *Nomioides* sp., y. *L. pauxillum* (Schenck) (Smith and Weller, 1989; Packer and Knerer, 1985), z. *Halictus resurgens* Nurse (Knerer, 1980), 1. *H. lanei* (Moure) (Packer et al., 2003), 2. *H. lutescens* Friese (Sakagami and Okazawa, 1985)*,* 3. *Thectochlora alaris* (Vachal) (Packer, unpublished data), 4. *Augochlorella semiaurata* (Spinola) Campos, 1980). The two arrows from point E denote the mean percentages for the two populations of this species studied, that from region III to the lower left, that from region VII to the upper right.

nies (see introduction). Sample means for each of the taxa included here, along with those previously published and a large number of Rophitines and a few other taxa (Packer, unpubl. data) are shown in Figure 3.

Five of the Chilean species had cooperative behaviour as the most common category. These were *Ca. dolator* with 81.5%, *Ca. species A* with 68.4%, *R. proxima* with 63.1%, *P. pississi* with 51.5% and *Ca. pygosinuatum* with 37% of interactions cooperative. All of these bees, except the last, also had low levels of aggression and avoidance behaviour (see Fig. 3). I suggest that the first 4 of these taxa have at least some nests that are communal, and they fit on the plot closest to those taxa known to exhibit this form of sociality.

None of the behaviourally unknown species had levels of aggression as high as *Co. chloris*. However, two other congeners had high levels of aggression, markedly higher than those of any of the other species. Thus, *Co. patagonica* and *Co. herbsti* had 40.4% and 40.3% of all interactions aggressive respectively. These data fit among the points representing samples for which a reproductive division of labour is known in Figure 3.

None of the species with unknown social organisation had levels of avoidance behaviour as high as *Penapis*. However, three of them had avoidance behaviour as the single most common behavioural category. Thus, *L. (D.) aricense, Ca. cuprellus* and *Co. melanocladius* had 48.8, 60.2, and

47.8 % of interactions resulting in avoidance behaviour respectively. For each of these species cooperative and aggressive interaction frequencies were very similar (Table 2B). A fourth species, *Co. corinogaster*, had a high frequency of avoidance behaviour (55.3 %) but a level of aggression substantially higher than its level of cooperative interaction, thus falling between the previous set of data points and those suspected of representing populations with a reproductive division of labour. *Caenohalictus pygosinuatum* had almost equal frequencies of all three behavioural categories but overall would seem to fit closer to the data points from solitary species than to any of the other groupings (Fig. 3).

Figure 3 indicates that there may indeed be separation of samples with a reproductive division of labour from those that are solitary and both of these from bees that are communal. There seem to be three clusters, one with low aggression but with cooperative interactions making up at least 50 % of all encounters, another with low levels of cooperation but with at least 40 % of interactions being aggressive and a third in which avoidance interactions are most common and aggressive and cooperative ones comparatively uncommon and generally approximately equal in frequency. These correspond, where known, to communal, semisocial or eusocial, and solitary social organizations respectively. The only data points that do not fit this scheme are for the mostly solitary species *Lasioglossum figueresi* and *D. mesillae* which are located among the taxa with a reproductive division of labour. It is possible that the higher levels of aggression in *L. figueresi* resulted from the fact that marked bees were used. It was shown earlier that marking can substantially increase the proportion of frontal encounters that result in an aggressive interaction in the solitary *P. toroi* (Packer, 2005). Studies of additional populations of *D. mesillae* would be worthwhile.

Across all samples, the proportion of all interactions that were "back and follow" did not correlate significantly with either aggressive or cooperative behavioural frequencies ($r_s = 0.147$, $p > 0.5$ and $r_s = -0.486$, $p > 0.1$ respectively; Fig. 4). However, back and follow did correlate significantly with the proportion of frontal encounters that resulted in avoidance ($r_s = 0.581$, $p < 0.05$, Fig. 4). Consequently, it would seem that back and follow, at least among the samples included here, is most likely to represent the attempt of the backing bee to escape from the advancing one rather than it being an attempt to lead and/or dominate it.

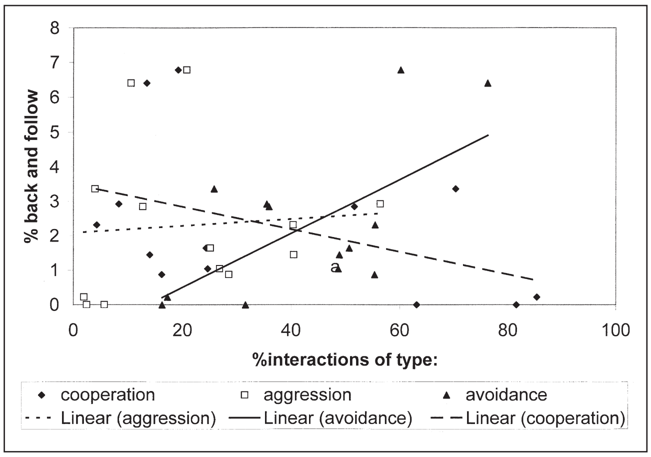

Figure 4. Proportion of all interactions that were "back and follow" versus the combined behaviours percentage cooperation, percentage avoidance and percentage aggressive when "back and follow" was not included. Each point represents results from one species (see tables for raw data).

Reproductive condition, age and size of the bees

This information was available for all samples except *Ca. pygosinuatum*, which was spoiled in storage, and two individuals of *Ca. cuprellus* that escaped. With the exception of two individuals of *Co. patagonica,* all bees included in the

Table 3. Ovarian, age and size variables for the samples of 14 Chilean halictines studied using the circle tube, coefficient of variation is given in parentheses, sample sizes are double those given in Table 1 for the number of circle tube experiments except where noted in the text.

	Total ovarian Development	proportion with whole oocyte	total wear	head width (mm)
P. toroi	2.21 (29.4)	90.0	3.1 (180.3)	2.15 (3.2)
R. mutabilis	2.31 (20.1)	62.5	7.63 (42.0)	2.48 (2.82)
Co . chloris	1.05 (87.2)	36.7	3.77 (100.2)	2.49 (4.95)
Co. melanocladius	1.86 (31.8)	80.0	5.4 (90.0)	2.26 (3.09)
Ca. dolator	1.54 (15.6)	37.5	8.63 (59.1)	2.24 (2.61)
Ca. cuprellus	1.61 (29.6)	30.0	5.34 (109.0)	1.76 (3.66)
Ca. species A	1.65 (41.5)	63.6	7.13 (61.7)	2.12 (1.50)
Ps. pississi	1.83 (30.3)	56.3	9.62 (79.1)	2.42 (5.93)
R. proxima	1.16 (30.3)	22.2	1.06 (60.6)	2.62 (3.28)
L. aricense	0.97 (55.8)	35.0	4.15 (56.0)	1.44 (2.92)
Co. corinogaster	1.80 (36.2)	44.4	0.89 (176.4)	2.54 (3.11)
Co. herbsti	0.26 (129.9)	0.0	1.38 (55.1)	2.28 (4.83)
Co. patagonica	0.71 (87.5)	16.7	1.38 (140.3)	1.92 (3.91)

analyses above had either some wing or mandibular wear or had some ovarian development. Consequently, it seems unlikely that all bees except these two were so newly emerged as to not have initiated nesting activities. All individuals of all bees whose spermatheca was found had mated. Information on reproductive status for the bees is shown in Table 3. It is expected that the samples considered as having a reproductive division of labour within some semisocial societies should have the highest coefficient of variation for ovarian development, resulting from some females inhabiting a nest solitarily with moderate ovarian development and foragers from multi-female nests having low levels of ovarian development. Samples with a reproductive division of labour should also have the greatest proportion of individuals that have no ovarian development at all. In terms of variance in ovarian development, the three species thought to have a reproductive division of labour ranked first, second and third out of 13, a significant result (Mann Whitney U test, $U = 30$, $p = 0.001$). For the proportion of bees with no ovarian development the same species rank first equal, 3rd and 4th, also a significant result (Mann Whitney U test, $U = 25.5$, $p < 0.05$). The reduced levels of ovarian development in the bees predicted to have a reproductive division of labour is not a result of their samples being comprised of a larger proportion of younger bees as they do not differ significantly in total wear scores from the other samples studied (Mann Whitney U test, $U = 23$, $p > 0.2$).

These data are consistent with the hypothesis that the more aggressive samples are of bees that have a reproductive division of labour among females with some individuals foraging but not having a commensurate reproductive output as a result of being subordinates in a multiple female nest. Conversely, the putatively communal or solitary species had more equivalent reproductive condition among females, indicating that they were all reproducing and that a reproductive division of labour was probably not in effect.

One might expect the species with a reproductive division of labour to have the highest coefficient of variation for body size. I removed *Ps. pississi* from this analysis because it was studied at two different locations and the bees in the two localities differed somewhat in size. Those species thought to have a reproductive division of labour did indeed rank first, second and third for variance in size; a significant result (Mann-Whitney U test, $U = 27$, $p = 0.001$).

Discussion

Given the difficulties in finding nests of most halictines and the time consuming activities required for even a cursory understanding of a population's social organisation from nesting observations, the possibility that the circle tube apparatus may provide a shortcut to estimating social organization is worth exploring. I have presented information on the relative proportion of various circle tube interactions among individuals for fourteen species of Chilean halictine bee, only three of which are, based upon other studies, thought to exhibit one or other of the various named categories of social organization. The circle tube behavioural profile of these samples fits expectations from their social organisations: one is highly cooperative, the second is comparatively aggressive and the third exhibits mostly avoidance behaviour. This pattern has also been found in other studies of bees with known social organizations (Fig. 3; McConnell-Garner and Kukuk, 1992).

Taking the above suggestions as a guideline and with reference to Figure 3, it is possible to suggest the probable social organisation of the remaining samples. Thus, *Co. herbsti* and *Co. patagonica* have behavioural profiles similar to that of their presumably semisocial congener *Co. chloris* and to other halictines known to have a reproductive division of labour, but within the eusocial context. Given the geographic distribution and phenology of these two species, they are southern Chilean taxa with one foraging period per year, it is likely that, if they do indeed have a reproductive division of labour, that they are semisocial rather than eusocial. But note that delayed eusociality, in which foundress females become queens when their worker daughters become active one year after they are produced (Packer, 1993), cannot be ruled out. *Caenohalictus dolator*, *Ca. species A, Ps. pississi* and *R. proxima* exhibit high levels of cooperation and low levels of aggressive and avoidance behaviours and thus can reasonably be suggested to be communal. Behavioural studies of the other two known species of *Ruizantheda* support this interpretation; see this paper for *R. mutabilis* and Michener and Lange (1958) for *R. divaricata* (as *Pseudagapostemon divaricatus*). Similarly, the only species of *Pseudagapostemon* for which behavioural data can be considered reliable, *Ps. cyanomelas* (Michener and Lange, 1958) is a communal bee. Michener et al. (1979) presented evidence that a Colombian species, *Caenohalictus eberhardorum*, had some communal nests, but with an average of only 1.8 females per colony. From the high rates of cooperative interaction found in the two *Caenohalictus* species noted above, I suspect that they may have larger mean colony sizes.

Caenohalictus cuprellus, Co. melanocladius and *L. aricense* exhibit behavioural profiles in which avoidance behaviours predominate. I believe this to be indicative of solitary behaviour. *Corynura corinogaster* and *Ca. pygosinuatum* may also be solitary. However, it is also possible, especially for the latter species, that they may have a low proportion of communal nests, with small colony sizes, as suggested for *Ca. eberhardorum* by Michener et al. (1979).

It is necessary to consider the extent to which my observations could be influenced by phylogenetic inertia (Orzack and Sober, 2001; Blomberg and Garland, 2002): closely related species may have behavioural profiles in circle tube arenas more similar to one another for phylogenetic reasons independent of actual differences in social organization. Evidence in support of this comes from the observation that the most aggressive behavioural profiles found herein involved species of the genus *Corynura* and that two of the most cooperative were closely related species within the genus *Caenohalictus*. A phylogeny is available that includes most of the genera/subgenera to which the species studied here belong (Danforth et al., 2004). Mapping the predicted social organizations for the species studied here onto an appropriate phylogeny (data not shown) does suggest a strong component of phylogenetic inertia in the results from circle tube experiments. However, they also indicate strong inertia in the social organizations exhibited by the bees that circle tube experiments hopefully reflect. Additionally, the phylogenies suggest that possession of communal social organization is the ancestral condition for the *Agapostemon* group of genera which includes *Ruizantheda, Caenohalictus* and *Pseudagapostemon* and that reversals to solitary behaviour may have occurred within *Caenohalictus*, as was demonstrated earlier within *Agapostemon* (Janjic and Packer, 2003).

Typification of the social organization of entire species based upon sociobiological investigations at a single locality, usually over a limited period of time, has been criticized on several grounds (Wcislo, 1997b). I am advocating the use of circle tube experiments as a preliminary tool for the prediction of the type of social organization of particular populations of particular species. Such an approach does not ignore the possibility of inter-, or even intrapopulational variation in the propensity for particular types of interaction (the uncooperative pair of *R. mutabilis* in figure 1 and the cooperative pair of *H. lanei* in Packer et al. (2003) are good examples of intraspecific variation). Variance in response among pairs of interactants is one measure of possible variation in behavioural profiles among individuals within or between populations. Population samples that seem atypical for the species or higher level taxonomic unit would then present themselves as being particularly worthy of more detailed investigation.

Other possibilities that must be considered when using bees of unknown nest affiliation in these experiments are that some of them may actually be nestmates (Packer et al., 2003) or that there is a subset of individuals that are cleptoparasitic upon the efforts of others (Packer, 1986). The probability of drawing two individuals from the same nest will depend upon the density of nests as well as the mean colony size at the site at that time. For workers of eusocial species, outlying cooperative pairs among a broader sample of aggressively interacting individuals could be detected as potential nestmates using genetic methods (see Darvill et al. (2004) for a similar approach to bumble bee nestmate discrimination).

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