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House mice and wood mice in and around an agricultural building

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INTRODUCTION

Wood mice *Apodemus sylvaticus* and house mice *Mus domesticus* are two of the commonest and most intensively studied British mammals. However, relative to the vast literature on non-commensal and laboratory house mice (Berry, 1981, 1991), and woodland wood mice (Flowerdew, Gurnell & Gipps, 1985; Flowerdew, 1991) little is known of the ecology of either species in and around farm buildings. Farm buildings and their surroundings are particularly important for house mice, which are a major stored-product pest (Meehan, 1984). In Britain they live largely indoors but also make limited use of hedges and fields in the summer (Rowe & Swinney, 1977; Montgomery & Dowie, 1993). Wood mice are very abundant on agricultural land, and occasionally use buildings (Green, 1979; Montgomery & Dowie, 1993; Tew & Macdonald, 1993).

Little is known of the degree to which wood mice and house mice overlap in their respective use of buildings and farmland. House mouse populations experimentally established by Tattersall, Smith & Nowell (1997) survived well in derelict farm buildings, but abundances of house mice in the areas surrounding the building were negatively correlated with abundances of wood mice. Data on commensal house mouse population dynamics, movements and interactions with other species are potentially useful for pest controllers, as well as providing an interesting comparison with non-commensal house mice and other rodents. Paradoxically, however, the fact that house mice are pests has hampered their study inside farm and other buildings, as owners suffering an infestation are reluctant to allow captured animals to be released for capture–mark–recapture (CMR) studies.

Here, I use CMR techniques to describe and compare naturally occurring populations of wood mice and house mice living in and around a piggery over a 1-year period, and assess the extent to which the two species overlap in their habitat use.

METHODS

Site

Trapping was carried out at Moorcopse piggery, a small, intensive pig unit in Berkshire, southern England comprising approx. 0.19 ha of buildings, plus about 0.1 ha of vegetation and concrete, bounded by fields and a small copse. The vegetation consisted of weedy species such as stinging nettles *Urtica urens*, red and white dead nettles *Lanium purpureum* and *L. album*, elder *Sambucus niger*, ground ivy *Glechoma hedereca*, brambles *Rubus fruticosus* and long grass.

The unit comprised 3 separate buildings: a small office, a farrowing house, and the main unit. Only the main unit was infested with house mice, despite the presence of pigs and food in the farrowing house. The main unit consisted of a weaning room, a large storage room, and 2 holding rooms. The storage room contained 3 large hanging funnels storing pig food, as well as sacks of food. Consequently there was a great deal of easily accessible food for the house mice in this area. For the first 4 trapping sessions of the study, cover was provided by large amounts of rubbish piled against the walls. Mice also nested in the cavity walls and in holes in the concrete floors.

Trapping

Eight Longworth live-trapping sessions were carried out between March 1990 and February 1991. Each trapping session lasted for 6 consecutive nights, and intervals between sessions were 6–9 weeks. A total of 62 traps was used outdoors, and 50 indoors. At least 55% of traps remained unsprung each morning. Trap layout was irregular due to constraints imposed by trapping in and around buildings, and by the day-to-day running of the piggery. Of the 50 indoor traps, 2 were placed in the farrowing house, 8 and 2 in the holding rooms, 5 in the waning room, and 33 in the storage area. Of the 62 outdoor traps, 17 were placed against an exterior wall and the remainder in vegetated areas 2–20m from the buildings.

Each animal captured was weighed, sexed and its

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reproductive condition noted. Females were considered to be in reproductive condition if they were perforate, obviously pregnant, or had exposed nipples, males if they had scrotal testes or if testes could be palpated into the scrotum. Wood mice were considered adult if they weighed >12g (Flowerdew, 1984), and house mice 11 g (Pelikan, 1981; Rowe, Swinney & Quy, 1983). Mice were individually fur-clipped until June 1990; however, house mice frequently had bald patches, bite wounds and torn ears, makeing fur-clipping or ear-tagging unreliable. Instead, they were individually marked by toe-clipping* under Home Office Licence. Comparison of newly and previously marked animals indicated that toe-clipping had no effect on recapture rates (Tattersall, 1992). In February, June and November 1990 there was sporadic poisoning with an unknown anticoagulant; although aimed at rats this also affected house mice, increasing trap mortality.

Abundance was estimated using the weighted mean method, following Begon (1979). Recapture rates for house mice were low (59% recaptured within 4 days, compared with 88% of wood mice), and their abundance is therefore likely to be overestimated relative to wood mice.

RESULTS

Abundance and demography

House mice were always more abundant than wood mice at Moorcopse (Fig. 1); densities of house mice reached the equivalent of 476/ha, while those of wood mice reached 343/ha (calculated indoors for house mice and outdoors for wood mice). Abundances of both species were highest in summer and lowest in winter.

Proportions of adult male and female wood mice trapped did not differ significantly from a 1:1 sex ratio in any session, but those of house mice did ($\chi^2 = 21.35$, d.f. $= 8$, $P < 0.01$). More male than female house mice were caught from March to November 1990, with an average of 60.4% of all captures being male. In December 1990 the proportion of male house mice caught was 40.4%, and this figure dropped to only 30.6% of captures in February 1991.

High proportions of reproductive house mice were present throughout the year (males 60–96%; females 45–80%), though there was some reduction in winter. Some male wood mice remained in reproductive condition throughout (31% in December), but females ceased reproduction over winter. For both species the ratio of adults to juveniles trapped changed significantly through the year ($\chi^2 = 16.03$, d.f. $= 6$, $P < 0.05$ for wood mice; $\chi^2 = 48.13$, d.f. $= 8$, $P < 0.001$ for house mice). Juvenile house mice were most prominent in July (43% of individuals), while juvenile wood mice were most prominent in September (34% of individuals).

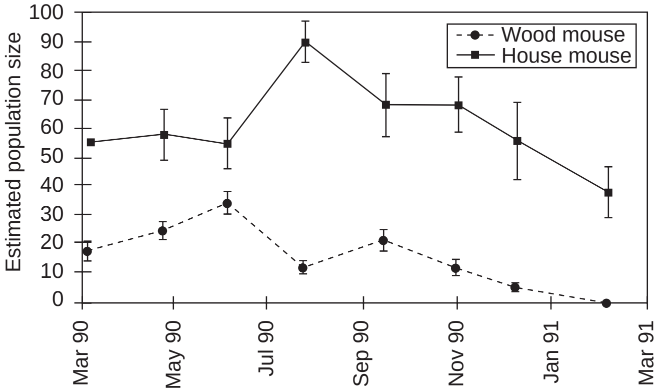

Fig. 1. Estimated population sizes (± SE) of house mice and wood mice at Moorcopse over a 1-year period. Estimates were calculated using the weighted mean method.

The proportion of new house mouse individuals which survived a 6- or 7-week interval between trapping sessions remained relatively stable (17–30%), albeit low, through the year. In contrast, the proportion of wood mice surviving between sessions was much more variable (13–67%), being highest in summer.

Habitat use by wood mice and house mice

Overall, only 20 (6.5%) of 307 house mice individuals were caught outside (monthly range: 0-9%). Among adults, there was no significant difference in the proportion of males and females found outside, either as numbers of captures or as individuals (overall, 10% of 128 males and 4% of 94 females occurred outdoors). There was no obvious seasonal trend in occurrence outdoors, but juveniles were found outside only in September (3 individuals) when the total number of juveniles was also high (25 individuals). However at peak juvenile numbers (34) in July, none were caught outside.

No wood mice were ever caught inside buildings, although one newly released animal ran into the farrowing house where it sheltered in a pile of straw; no house mice were caught in the farrowing house (regular inspection of the interior of the building for droppings and food remains confirmed the lack of house mice). There was also a clear difference in habitat choice outside the buildings: house mice were more likely than wood mice to be caught within 1m of buildings ($\chi^2 = 100$, d.f. $= 1$, $P < 0.001$). Most (78%) of the 36 captures of house mice outside occurred within 1m of an outside wall, but this was true for only 12% of 416 captures of wood mice. Of the house mice captured outside, adult females were more likely than adult males to be captured over 1 metre away from an infested building (85.7% of captures *vs* 11.5%; $\chi^2 = 15.3$, d.f. $= 1$, $P = 0.0005$).

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* Editor's note: The Ethical Committee of the Zoological Society considers that toe-clipping is no longer acceptable as a routine procedure for marking animals.

DISCUSSION

Wood mouse populations in woodland usually peak in autumn and winter, decline in spring, and remain stable in summer (Flowerdew, 1985; Wilson, Montgommery & Elwood, 1993). In contrast, the wood mouse population at Moorcopse was greatest in summer and lowest in winter. Densities of wood mice in the scrubby vegetation at Moorcopse were unusually high – up to the equivalent of 342/ha in summer. In urban areas wood mice also reach high densities (70–80/ha) in scrub (Dickman & Doncaster, 1987). Scrubby areas may act as sinks for spring- and summer-born young. The fluctuations of wood mice at Moorcopse suggest that animals moved into the area in the spring, bred, and mostly died or moved away in winter (Tattersall, 1992). Wood mice probably originated in a patch of woodland next to Moorcopse, and in nearby hedgerows.

Regular seasonal changes are not so well established for house mice as for wood mice. Breeding in house mice tends to be seasonal in outdoor populations (Berry, 1964; Newsome, 1969*a*, *b*; Stickel, 1979; Pelikan, 1981; Bomford, 1987), but aseasonal indoors (Adamkzyk & Ryskowski, 1965; Bergeron & d'Astous, 1980), as it was at Moorcopse. Despite continuous breeding, abundance at Moorcopse decreased during winter, although towards the end of the study this was accompanied by a reduction in food and cover, as parts of the piggery were cleared. Rowe & Swinney (1977), and Rowe *et al.* (1983), did not report seasonal changes in abundances in farm buildings and on arable land, but Rowe, Quy & Swinney (1987) found a reduction in population size in mid-winter, with smallest numbers in January and February. Longer-running studies in a wider range of building types are needed to establish the relative importance to population dynamics of seasonal changes in temperature, changes in resource availability, and control measures such as poisoning.

Although house mice readily live outside (Berry, 1981) the majority of individuals at Moorcopse, as on a number of other British farms (Rowe & Swinney, 1977), lived indoors, with a small proportion of the population occurring outdoors in some sessions. Rowe & Swinney (1977) captured most house mice outside in summer and autumn, and Zejda (1975) captured house mice outside in southern Moravia between February and October. The majority of outside captures at Moorcopse were between April and September, but some animals were still caught outside in December.

Pelikan (1981) described house mice moving out of buildings into fields in spring, and back again in winter. At Moorcopse, however, $<10\%$ of animals were captured outdoors in any one session, and there were no seasonal trends. Correlation of the movement of house mice out of buildings with high population density has been reported (Anderson, 1978; Pelican, 1981), but this did not seem to be the case at Moorcopse, where the proportion captured outside remained relatively constant. Furthermore, as the majority of captures of house mice out of buildings were in traps against exterior walls, and in some cases animals were recaptured indoors, they may represent movements within a home range, rather than emigration.

Rowe & Swinney (1977) caught more mice outdoors in hedgerows near to buildings than in more distant hedgerows, but more females than males were caught in the more distant hedgerows. Predominance of males near buildings, and females in far hedgerows was also found by Reimer & Petras (1968). The situation was similar at Moorcopse, where a significantly greater proportion of females than males were found more than 1 m away from the buildings. Myers (1974) showed that feral females, particularly those in reproductive condition, dispersed more than males. As females are more important than males in starting a new colony, a reasonable assumption would be that they are the principal colonizers.

At Moorcopse there was no evidence of an interaction between house mice and wood mice in the form of a negative correlation between population sizes of the two species. Outdoors, the two species were spatially separated, but whether this was the result of habitat choice or competition requires manipulative studies. Wood mice did not colonize the house mouse-free farrowing house, although one wood mouse was seen just inside the door to the farrowing house. Similarly, proportions of house mice captured out of the buildings did not vary with wood mouse abundance. The clear spatial separation of the two species suggests that wood mice are unlikely to be exposed to rodenticides inside buildings. The small numbers of house mice outside indicate that, in British farm buildings, rodenticides placed outdoors will be of little benefit for controlling an existing infestation.

In summary, at Moorcopse the picture was of a very dense, continuously breeding, population of house mice, with high turnover. Indeed, many animals were scarred and wounded, perhaps the result of intraspecific fighting. Although they lived at higher than normal densities at Moorcopse, wood mice were less numerous than house mice, bred seasonally, and displayed no evidence of fighting. There was apparently little overlap in habitat use by the two species: house mice were predominantly caught indoors or adjacent to external walls, but wood mice were only captured outdoors.

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Does the method of set-aside establishment affect its use by wood mice?

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INTRODUCTION

The set-aside scheme, whereby land is removed from arable production, is primarily aimed at manipulating agricultural surplus within the European Community, but has potentially great consequences for biodiversity and wildlife management (Feber *et al*., 1995; Macdonald *et al*., 1998; Sotherton 1998). Managed inappropriately, set-aside could prove environmentally damaging by reducing biodiversity and directly harming certain species, such as ground-nesting birds (Thompson, 1993). Conversely, appropriately managed set-aside could foster desirable ecological communities, with enhanced populations and biodiversity.

In Britain, farmers are encouraged, but not obliged, to manage their set-aside in an environmentally friendly manner (MAFF, 1995). However, there has, as yet, been insufficient research to establish clearly what features of set-aside are good for wildlife, and for mammals in particular. Tattersall *et al.* (1997) failed to demonstrate differences in abundances of small mammals on naturally regenerated and sown set-aside, but they used live-trapping, which is a relatively insensitive means of measuring habitat preferences.

We used radio-tracking to investigate the impact of one aspect of set-aside management, the use of different seed mixtures in its establishment, on habitat use by a common arable small mammal, the wood mouse *Apodemus sylvaticus*. The method of set-aside establishment, and the resulting vegetative composition and structure, is likely to affect factors such as food availability and cover from predators, both of which have been demonstrated to alter wood mouse behaviour (Tew, Macdonald & Rands, 1992; Plesner Jensen & Honess, 1995; Tew, Todd & Macdonald, in press). We therefore expected the mice to behave differently in set-aside sown with different seed mixtures.

METHODS

Sites and set-aside establishment

We tracked wood mice on 2 arable farms: ZENECA Agrochemicals' Jealott's Hill Farm (Berkshire) and the Royal Agricultural College's Eysey Manor Farm (Wiltshire). Study sites (15 ha at Jealott's Hill, 25 ha at Eysey), encompassed set-aside bounding 2 cereal fields on each farm (winter wheat and oats at Jealott's Hill, winter wheat and barley at Eysey). Also included in our study areas were hedgerow (both sites), and at Jealott's Hill only, beetle-bank (a 1 m strip of rough grassland running through the crop centre), fallow and pasture.

Set-aside at Jealott's Hill was established in autumn 1996 using 2 species-rich mixes of grasses and native forbs (*Poa annua* at 6% seed weight plus 15 broadleaf species, and 8 species of grasses at 80% plus 15 broadleaf species) sown in the 10 m next to the crop and the 10 m next to the hedge at densities of 4 kg/ha and 10 kg/ha, respectively. Vegetation in 1997 was heavily dominated by wheat (from grains spilled at harvest), and for our purposes the 2 mixes are considered together. Set-aside at Eysey was established on 20 m wide margins and an adjoining 5 ha block in autumn 1995. A considerably simpler mixture was used, consisting of 6 agricultural grasses (including perennial rye grass to ensure quick establishment of cover) and 2 clovers, sown at a rate of 24.7 kg/ha, more than twice the highest rate at Jealott's Hill.

Radio-tracking

We used data for 18 male wood mice. Nine were followed at Eysey during June and July 1996 and 1997, and 9 at Jealott's Hill during late May and June 1997.

We captured wood mice using aluminium Longworth live-traps and following standard procedures (Gurnell & Flowerdew, 1990), and retained males larger than 20 g for radio-tracking. Animals were collared under light anaesthetic (under Home Office licence to FHT) using Metophane (C-vet). Transmitters (Biotrack U.K. Ltd) were integrated into self-locking cable tie collars; they weighed 2 g, operated at 173 MHz, and their batteries lasted about 4 weeks. Once sufficient fixes had been taken we endeavoured to recapture the animal and remove its collar.

We used a Mariner receiver (Mariner Radar, Lowestoft) and hand-held 5-element Yagi aerial to track the mice continuously through the night, usually from 21:00 to 04:00, although we did not always gather a full night's worth of data. On the first night, the first 4 h of data gathered for a mouse were discarded, to allow it to become habituated to the presence of the tracker. For each fix, the animal was located to within 5 m, using signal strength and direction as a guide. We were able to gauge our position in the dark, and to take fixes, by referring to a mapped grid of flexible fibre-glass canes located in the field at 50 m intervals. Each cane was individually colour-coded with reflective tape which could be seen in the light of our head-torches.

Vegetation surveys

In July 1997 the set-aside and crop vegetation in the 2 study areas was surveyed. We measured set-aside height and cover, and recorded the dominant species in 1 m^2 quadrats, set 5 m and 15 m into the set-aside from the field boundary. The vegetation was sampled at 150 m intervals, resulting in 34 set-aside samples at Eysey and 10 at Jealott's Hill. We also measured crop height 10 m into the crop adjacent to set-aside, resulting in 10 samples at Eysey and 20 at Jealott's Hill. Cover was estimated by eye for bare soil, grasses and herbs, and divided into 5 categories: 1 (< 5%), 2 (5–25%), 3 (25–50%), 4 (50–75%) or 5 (75–100%) (Bullock, 1996).

Analysis

We only used data from animals for which we had a minimum of 92 fixes (Tew, 1989), collected over 3 nights (including the second half of the first night on which a mouse was tracked). Home-range sizes were calculated using the RANGES V package (Kenward & Hodder, 1996). We estimated home-range sizes using the concave polygon (CP) method (Kenward, 1987) and, for comparison, the minimum convex polygon (MCP) method (Mohr, 1947). Neither of these methods is affected by autocorrelation (Harris *et al.*, 1990). In RANGES we chose the default 'selected edges only' option, with edge lengths set at the default 0.5% range width, when calculating CP ranges, and for the MCP method we used the default 'outer 100% edges' option. Habitat composition of home ranges, and number of fixes in each habitat type, were also determined using the

RANGES V package. We entered maps of our sites (using accurate farm maps which we ground truthed), and the habitats within them, as edge co-ordinates to the nearest metre.

To investigate habitat preferences, we used compositional analysis (Aebisher, Robertson & Kenward, 1993), which takes into account the relative proportions of utilized and available habitat, and is not biased by non-independent data. At each site, we compared habitat use (as numbers of fixes in each habitat category) with habitat availability (as the proportion of each habitat category present within an animal's home range, estimated using the CP method). We divided habitats into 3 categories: 'crop', 'set-aside' and 'other' (hedgerow at both sites, pasture and fallow at Jealott's Hill). Although there were differences between sites in the habitats available to wood mice, in practice the mice used these habitats so infrequently (for example, they never went into the pasture, and only 4 fixes were recorded in fallow) that the differences were extremely unlikely to bias our results. Previous analysis (Macdonald *et al.*, 1998) showed that male wood mice at Eysey did not differ in their use of set-aside margins and the 5 ha block, and these were therefore considered together. We used data from CP home ranges because these are more conservative than MCP ranges and do not include larger areas which are never visited by the study animals (Harris *et al.*, 1990).

Compositional analysis was used to produce a significance level for the overall deviation from random habitat use and, for each individual, a preference value for each habitat. For pair-wise comparisons between habitats, we performed a Wilcoxon signed rank sum test on the differences between the 2 preferences.

RESULTS AND DISCUSSION

There were no statistically significant differences in home-range sizes at Jealott's Hill and Eysey (Table 1); larger means at Jealott's Hill were predominantly due to two males with MCP ranges of 9.5 ha and 8.6 ha. For all 18 animals together, a mean of 103.9 ± 14.9 fixes was gathered, over 4 ± 0.9 days. Overall range size estimated using the MCP method was 2.96 ± 2.5 ha, while CP range size was 2.43 ± 2.4 ha. These ranges are similar to those previously obtained by continuous radio-tracking in cereals (Tew & Macdonald, 1994; Tew, Todd & Macdonald, in press).

Table 1. Home-range sizes and fix sample information for Eysey and Jealott's Hill farms

Site	*n*	Mean fixes ± SD	Mean days ± SD	Mean MCP ± SD (ha)	Mean CP ± SD (ha)
Eysey	9	106.6 ± 15.7	4.3 ± 0.9	2.1 ± 1.3	1.9 ± 1.1
Jealott's Hill	9	101.2 ± 14.5	3.7 ± 0.9	3.8 ± 3.2	3 ± 3.1

There were significant differences between Eysey and Jealott's Hill set-aside in vegetative height, cover and species richness, but not in the proportion of cover provided by grass and herbaceous species (Table 2). Vegetation at Eysey was taller and provided more cover than vegetation at Jealott's Hill, but contained fewer species. Crop at Eysey was significantly taller than at Jealott's Hill (Table 2) but this may have been a function of the timing of surveying, which was later at Eysey.

On average, wood mice at Eysey had a high preference for 'other' habitat, and avoided 'set-aside', while at Jealott's Hill they preferred 'set-aside' and avoided 'crop' (Fig. 1). However, only at Eysey was there a statistically significant deviation from random habitat use overall ($P = 0.003$); at Jealott's Hill mice used habitats within their home ranges at random relative to their availability ($P = 0.7$). Pairwise comparisons between habitats were not significant at either site.

Although we were unable to make a direct comparison of the use of different seed mixtures by wood mice, we can none the less infer something of their preferences from their relative use of set-aside, crop and other habitats. We hypothesized that set-aside margins established using different seed mixtures and sowing rates would differ in their vegetative characteristics, and that wood mice would detect and respond to these differences. We expected that the mice would prefer set-aside margins which provided them with greater cover. While different establishment methods did indeed result in predictable vegetative characteristics – richer seed mixtures resulted in greater species diversity, and lower sowing rates resulted in lower cover – the mice avoided taller, denser set-aside vegetation relative to crop and other habitats. As they did not avoid the shorter, species-rich but sparse set-aside, we suggest that of the two types examined, this was the more favourable.

Disentangling the reasons why the Jealott's Hill set-aside was apparently preferred is difficult, but none the

Table 2. Vegetation characteristics at Eysey and Jealott's Hill. Significance levels to compare the two sites were obtained with a two-tailed *t*-test, variances assumed unequal. Errors are standard errors. *** = $P < 0.001$, NS = non-significant

	Set-aside						Crop	
Site	*n*	Height (m)	Cover (%)	Grass (%)	Herb (%)	Species richness	*n*	Height (m)
Eysey	34	1.0 ± 0.1	91 ± 11	62 ± 25	31 ± 27	5 ± 0.9	10	0.8 ± 0.16
Jealott's Hill	10	0.6 ± 0.1	75 ± 11	45 ± 26	31 ± 19	17 ± 3.0	20	0.7 ± 0.16
	***	***	NS	NS	***			

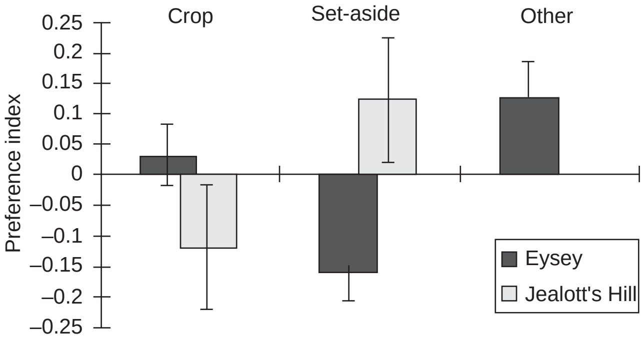

Fig. 1. Mean (± SE) preference indices for crop, set-aside and other habitats before harvest and topping at Eysey and Jealott's Hill. A positive value indicates that a habitat is used more than expected (i.e. is preferred) while a negative value indicates that a habitat is used less than expected.

less interesting as it raises wider questions about how animals use habitats within a mosaic landscape. One possibility is that the crop and other habitats were more attractive relative to set-aside at Eysey than at Jealott's Hill. Tew & Macdonald (1993) demonstrated an increased risk of predation resulting from the lack of cover following harvest; the *c*. 10 cm taller crop at Eysey may have provided more protection than the crop at Jealott's Hill, although this seems unlikely as both crops were cereals (oats and winter wheat at Jealott's Hill, winter wheat at Eysey). There is no evidence that wood mice differentiate between different arable crops (Todd, Tew & Macdonald, in press). More importantly, at Eysey 'other habitat' contained more hedgerow and less fallow and pasture than at Jealott's Hill, and hedgerow is a favourite habitat for arable wood mice (Tew, Todd & Macdonald, in press).

Alternatively, if we assume that the behaviour of the mice was a result of the different vegetation characteristics arising from the seed mixtures, then it appears that the mice were selecting set-aside on the basis of plant diversity rather than protection from predators. On grass margins in Oxfordshire, Plesner-Jensen & Honess (1995) found that vegetation height had an important influence on the capture of small mammals, with more captures in plots with tall (60 cm) rather than short (5 cm) grass. Set-aside at both our sites was more than 60 cm tall, on average, and so even the relatively short sward at Jealott's Hill may have provided sufficient cover. More diverse vegetation might provide more food resources for wood mice, by providing seeds over a longer fruiting period, and by harbouring more invertebrates (Feber *et al*., 1995).

We have shown that wood mice used set-aside sown with different seed mixtures to different extents relative to the other habitats available to them. This demonstrates that set-aside can differ in its attractiveness to small mammals, but also sounds a cautionary note in jumping to conclusions about the impact of farm management practices on wildlife.

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The social calls of Kuhl's pipistrelles *Pipistrellus kuhlii* (Kuhl, 1819): structure and variation (Chiroptera: Vespertilionidae)

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Abstract

The aim of this study was to describe the structure of social calls produced by Kuhl's pipistrelles *Pipistrellus kuhlii*. Bats foraging around street lamps in the Campania region, Southern Italy, were recorded. Calls were produced by bats during chases and, similarly to those of *Pipistrellus pipistrellus*, were probably used to repel conspecifics from hunting sites. Calls often had three components, lasted on average 34 ms and contained most energy at about 17 kHz. A positive correlation was found between the frequencies of maximum amplitude of echolocation and social calls. Social calls from two adjacent populations differed in their peak frequencies, and possible hypotheses for this variation are given. The structure of *P. kuhlii* social calls was compared with those of the 45 and 55 kHz *P. pipistrellus* phonic types. Kuhl's pipistrelle calls lasted longer and showed lower values of minimum and peak frequencies. These differences can help to discriminate between field recordings of *P. kuhlii* and *P. pipistrellus* where the two species occur sympatrically.

Key words: Chiroptera, communication, *Pipistrellus kuhlii*, social calls

INTRODUCTION

The Kuhl's pipistrelle *Pipistrellus kuhlii* (Kuhl, 1819) is a small vespertilionid with a body mass of 5–10 g and a forearm of 31–37 mm (Schober & Grimmberger, 1997). It is distributed through south and south-west Europe, including the Balkans and the Mediterranean islands, extending northwards to Switzerland, France and Austria, eastwards to the Caucasus (Schober & Grimmberger, 1997) and southwards to Africa (Corbet, 1978).

Pipistrellus kuhlii echolocation search calls are 8–12 ms long (Schnitzler *et al*., 1987) and consist of a short frequency-modulated (FM) component followed by a terminal part of almost constant frequency (CF). The terminal part corresponds to the frequency of highest energy, generally 35–40 kHz (Schnitzler *et al*., 1987; Ahlén, 1990; Barataud, 1996). Call structure varies depending on whether the bat flies in cluttered or uncluttered habitats (Kalko & Schnitzler, 1993).

Like many other bat species, bats from the genus *Pipistrellus* also emit vocal signals for communication (Ahlén, 1981, 1990; Miller & Degn, 1981; Fenton, 1985). These calls may serve different functions: *Pipistrellus pipistrellus*, for example, produces social calls in an aggressive context (Barlow & Jones, 1997*a*), songflight calls by males to attract females to the roost in the mating season (Lundberg & Gerell, 1986; Gerell-Lundberg & Gerell, 1994; Barlow & Jones, 1997*b*), and distress calls to attract conspecifics and probably to encite other bats to mob predators (Russ, Racey & Jones, 1998).

Social calls can be used to discriminate between different pipistrelle species: significant differences exist between the structures of advertisement calls of *P. pipistrellus* and *Pipistrellus nathusii* (Barlow & Jones, 1996), and even the two cryptic species of *P. pipistrellus*

(Jones & Parijs, 1993; Park, Altringham & Jones, 1996; Barlow, Jones & Barratt, 1997; Barratt *et al*., 1997) can be separated by examining the structure of their songflight and social calls (Barlow & Jones, 1997*b*).

Although *P. kuhlii* is known to emit social calls (Ahlén, 1990; Barataud, 1996), no detailed description of these signals has been reported so far.

In the present study we: (1) provide a detailed analysis of social calls emitted by foraging Kuhl's pipistrelles, (2) test the hypothesis that bats emitting echolocation calls with higher peak frequencies also produce social calls with higher frequencies of maximum energy, (3) investigate whether there are geographical differences in social call structure between two populations < 100 km apart.

We also discuss the possibility of discriminating *P. kuhlii* in flight from other pipistrelle species producing similar echolocation calls (*P. pipistrellus*, *P. nathusii*) by examining social calls.

METHODS

Field recordings

From August to October 1998, we recorded social calls emitted by foraging bats in the Campania region, Southern Italy (14°15′E, 40°50′N). *Pipistrellus kuhlii* is the most common bat species in this area. Almost all calls analysed were recorded in the Benevento and Naples provinces, about 70 km apart. Only 1 call was recorded in the Salerno Province.

We made recordings near street lamps, where *P. kuhlii* commonly forages (Haffner & Stutz, 1985/6) and seems to take advantage of group hunting (Barak & Yom-Tov, 1989).

The habitats occurring at the recording sites in the Benevento province were arable land, olive groves and vineyards, while in the Naples province we carried out recordings in urban areas and suburbs with a mosaic of buildings, small cultivations and gardens. In order to avoid pseudo-replication (Hurlbert, 1984), we made recordings at lamps located at least 2 km apart, and considered for analysis only 1 call sampled at each site. In this way only 1 signal for each bat was represented in the sample.

Recordings were made via the high-frequency output of an S25 bat detector (Ultra Sound Advice, London) connected to a Portable Ultrasound Processor (Ultra Sound Advice, London) which sampled at a rate of 448 kHz and time-expanded (10) a 2 s sequence of calls. The resulting sequence, lasting 20 s, was then replayed and recorded on Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C. The S25 microphone has a sensitivity of -57 ± 3dB (ref. 1 V/lbar) from 20 to 120 kHz. We could compare *P. kuhlii* social calls with those by British *P. pipistrellus* described by Barlow & Jones (1997*b*) as an identical equipment was used in both studies.

Sound analysis

The recordings were analysed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). We adopted a sampling frequency of 44 100 samples/s, with 16 bits/sample; a 512 pt. FFT with a Hamming window was used for analysis.

For each social call, we measured the following parameters: the number of components to the call (*nocomp*), the total duration of the call (*totdur*), the minimum (*fmin*) and maximum (*fmax*) frequencies of the call, and the mean frequency of highest energy (*freq*) by measuring the peak frequency of each component and calculating the average. Duration was measured from oscillograms, and all other parameters were taken from sonagrams.

Identification of *P. kuhlii*

Pipistrellus kuhlii is the most abundant bat species in all localities where we carried out recordings. Its presence in 10 of them was also confirmed by hand-netting the bats soon after ultrasound recordings had been taken.

In all cases, we attributed the social calls recorded to *P. kuhlii* only when they were produced by bats emitting FM–CF echolocation calls with a frequency of maximum amplitude of 36–41.5 kHz. *Pipistrellus kuhlii* echolocation calls show a wider range of peak frequencies (Zingg, 1990); however, the criterion we adopted eliminated any possible risk of confusion with the 45 kHz phonic type of *P. pipistrellus,* as the lowest values of peak frequencies reported for this species from continental Europe (Zingg, 1990) and Great Britain (Vaughan, Jones & Harris, 1997) is 41.6 kHz, and Zingg (1990) indicates values of peak frequency for *P. kuhlii* and *P. pipistrellus* which overlap within the range 41.6–44.8 kHz.

Usually the bats considered for the present study emitted echolocation calls peaking below 40 kHz. No individuals of the 45 kHz *P. pipistrellus* phonic type were captured in the study areas.

Pipistrellus nathusii also emits echolocation calls similar to those produced by *P. kuhlii* (Zingg, 1990); however, this species is uncommon in Southern Italy (Lanza, 1959) and is not known to occur in Campania.

Statistical analysis

An Anderson–Darling test applied to the parameters measured on social calls and on peak frequencies of echolocation calls revealed that they did not conform to normal distribution, and normality was not obtained through data transformation. Therefore, we used nonparametric tests for univariate analyses: a 2-tailed Mann–Whitney test was applied to test for differences between medians of each parameter, and a Spearman's rank coefficient was used to explore correlation between the peak frequencies of echolocation and social calls. As

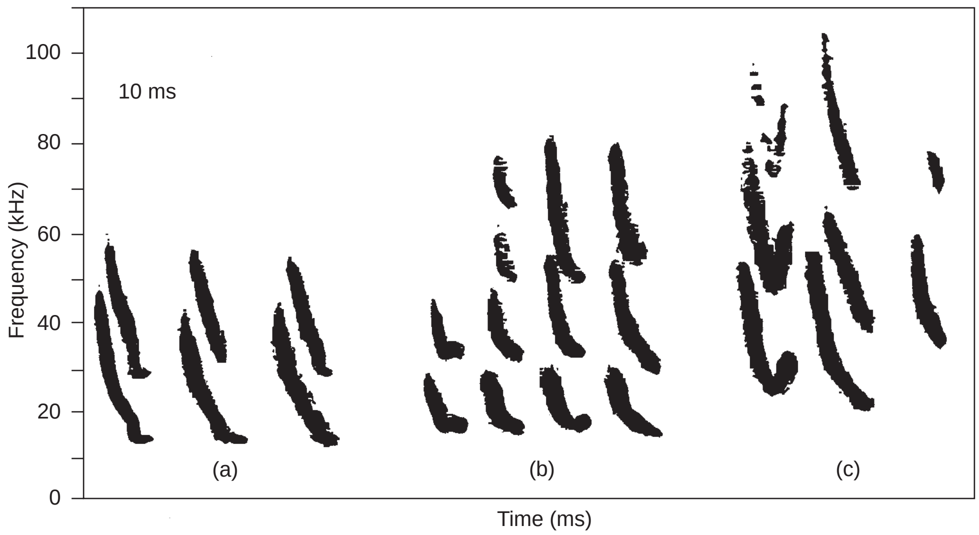

Fig. 1. Sonagrams of a social call of (a) *Pipistrellus kuhlii*, (b) the 45 kHz phonic type, and (c) the 55 kHz phonic type of *P. pipistrellus*.

multivariate techniques are robust to departures from normality (Dillon & Goldstein, 1984), we also employed a quadratic discriminant analysis with cross validation to try to separate call samples from the 2 study areas and a MANOVA to obtain values for Wilk's λ. Analyses were performed with Minitab release 11.0. In all tests, values of $P < 0.05$ were considered significant.

RESULTS

P. kuhlii social calls

Pipistrellus kuhlii foraged at all recording sites, as confirmed by the numerous feeding buzzes (Griffin, Webster & Michael, 1960) they produced. Two or more bats flew by the street lamps, and in most cases we could observe them chasing while emitting social calls. Because of their low frequencies, these signals were distinctly audible to the unaided ear. A total sample of 50 social calls, each from a different bat, was analysed.

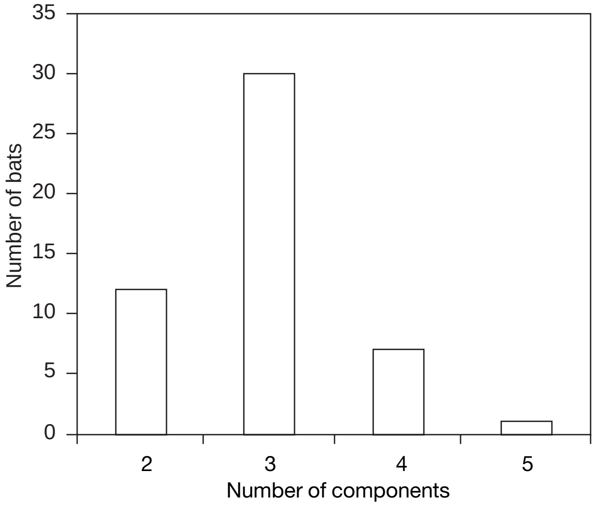

Fig. 2. Number of components of social calls emitted by 50 *P. kuhlii*. Only one call from each bat was examined.

Figure 1 (a) shows a typical social call of *P. kuhlii*. Thirty out of 50 Kuhl's pipistrelles produced three-component social calls, although calls with two, four and, once, five components were recorded (Fig. 2). Calls lasted approx. 34 ms, peaked at 16.6 kHz and showed a mean frequency bandwidth of 26.7 kHz (Table 1).

We found a significant positive correlation between *freq* and the peak frequency of the echolocation call preceding the social call ($n = 40$, $r_s = 0.342$, $P < 0.05$). The median values of peak frequency measured on the echolocation calls preceding the social calls did not differ significantly between Naples and Benevento (nNaples= 27, nBenevento = 13, $t = 126.0$, $P = 0.156$).

Of all variables measured on calls from the study areas, only *freq* showed a significant difference, i.e. bats from Benevento emitted social calls peaking at frequencies about 2 kHz higher than those from Naples (Table 2). A quadratic discriminant analysis with cross validation carried out on samples from the Benevento ($n = 22$) and Naples ($n = 27$) provinces could correctly classify 61.2 % of calls, but the model was not significant (Wilk's $\lambda = 0.79$, $F_{5,43} = 2.2$, $P = 0.07$).

Comparison between *P. kuhlii* and the two phonic types of *P. pipistrellus*

Sonagrams of social calls of *P. kuhlii*, and of the 45 kHz and 55 kHz *P.pipistrellus* phonic types all show more than one component (Fig. 1a–c). Like *P. kuhlii*, the 55 kHz *P. pipistrellus* phonic type also produces more frequently calls of three components, while the 45 kHz *P. pipistrellus* phonic type generally emits four-component calls (Barlow & Jones, 1997*b*). Social calls of *P. kuhlii* are longer than those of both phonic types of *P. pipistrellus*, and show lower values of *fmin* and *freq*

Table 1. Social call parameters from the 50 Kuhl's pipistrelles recorded in the present study, and from 24 bats of the 55 kHz phonic type and 22 bats from the 45 phonic type of *P. pipistrellus* after Barlow & Jones (1997*b*). Q1 and Q3 are the lower and upper quartiles

	Pipistrellus kuhlii					*Pipistrellus pipistrellus*	
Parameter	Mean ± SD	Range	Median	Q1	Q3	55 kHz phonic type (mean ± SD)	45 kHz phonic type (mean ± SD)
Totdur (ms)	34.4 ± 8.31	21.2–56.3	33.5	28.0	39.0	24.9 ± 3.48	30.5 ± 3.46
fmin (kHz)	11.5 ± 0.81	9.8–13.1	11.4	10.9	12.0	16.6 ± 1.80	15.2 ± 1.93
fmax(kHz)	38.2 ± 4.50	29.5–49.2	38.5	35.7	40.8	39.5 ± 5.69	34.4 ± 4.91
freq (kHz)	16.6 ± 2.70	13.5–24.6	16.2	14.2	18.3	20.8 ± 1.56	19.8 ± 2.53
Nocomp	2.9 ± 0.68	2–5	3	2.8	3.0	3.0 ± 0.42	4.0 ± 0.49

Table 2. Social call parameters of Kuhl's pipistrelles from the provinces of Naples ($n = 27$) and Benevento ($n = 22$), values of Mann–Whitney *t* statistic and corresponding levels of significance *P*. For each parameter the value from the Benevento sample is given below that from the Naples sample

Parameter	Mean ± SD	Range	Median	Q1	Q3	*t*	*P*
Totdur (ms)	33.5 ± 7.04	22.6–52.0	33.8	26.6	38.8	293.5	0.952
	34.4 ± 8.72	21.2–53.0	32.8	27.8	39.5		
fmin (kHz)	11.4 ± 0.79	10.3–13.1	11.4	10.9	12.0	267.0	0.543
	11.5 ± 0.86	9.8–13.1	11.4	11.1	12.0		
fmax (kHz)	37.4 ± 4.91	29.5–48.6	37.3	33.4	40.7	229.5	0.117
	39.2 ± 3.93	31.7–49.2	39.0	35.7	41.8		
freq (kHz)	15.7 ± 2.20	13.6–22.9	15.4	14.0	17.3	161.5	0.003
	17.8 ± 2.84	13.9–24.6	18.1	15.6	18.9		
Nocomp	2.8 ± 0.56	2–4	3.0	2.0	3.0	253.5	0.387
	3.0 ± 0.79	2–5	3.0	2.7	3.2		

(Table 1). The difference in *fmax* appears considerable (about 4 kHz) only between *P. kuhlii* and the 45 kHz phonic type.

DISCUSSION

Pipistrellus kuhlii social calls

The social calls of *P. kuhlii* we describe were all emitted during chases in foraging grounds, and are therefore probably equivalent in function to those observed in *P. pipistrellus*, i.e. they are used for food patch defence (Barlow & Jones, 1997*a*).

We also noticed that social calls were more frequent on cold nights, probably because insect density is reduced by low air temperatures (Williams, 1961). At low insect densities, *P. pipistrellus* performs most chases (Racey & Swift, 1985) and produces higher social call rates (Barlow & Jones, 1997*a*).

Our study shows that bats emitting echolocation calls at higher peak frequencies also produce social calls with a higher frequency of maximum amplitude. This relationship does not occur in *P. pipistrellus* (Barlow & Jones, 1997*b*).

As our data show no difference between echolocation call frequencies from the Naples and Benevento areas, the microgeographical variation observed in *freq* cannot be explained by the above discussed relationship between peak frequencies of echolocation and social calls. This difference could be the result of random effects of natural variation. A further hypothesis, however, is that the Kuhl's pipistrelles from Naples emit social calls at lower frequencies as a result of an acoustic adaptation process aimed to reduce attenuation and degradation of propagating calls in a complex habitat such as the urban area, while bats from Benevento produce social calls with higher frequency as they forage in simpler, uncluttered habitats. The acoustic adaptation to habitat structure (Morton, 1975; Wiley & Richards, 1978) is known to occur at a microgeographical scale in several bird and mammal species (e.g. Galeotti, Appleby & Redpath, 1996; Slobodchikoff, Ackers & Van Ert, 1998).

Comparison between *P. kuhlii* and the two phonic types of *P. pipistrellus*

Social calls of *P. kuhlii* show lower values of *freq* and *fmin* than those by both British *P. pipistrellus* phonic types (Barlow & Jones, 1997*b*). These differences are also likely to exist where these species occur sympatrically, as social calls of *P. pipistrellus* from France, Portugal, Sweden, are very similar to those described for Britain (G. Jones, pers. obs.). Values of *freq* are inversely related to body size between species, as *P. kuhlii*, which calls at the lowest frequencies, is larger than *P. pipistrellus*, and the 55 kHz *P. pipistrellus*, which calls at the highest frequencies, is smaller than the

45 kHz phonic type (Jones & Parjis, 1993; Barlow & Jones, 1997*b*). Larger bat species bear larger vocal tracts, and tend to produce echolocation calls at lower frequencies than smaller species (Barclay & Brigham, 1991; Jones, 1995; Vaughan *et al*., 1997). The negative relation to body size observed in pipistrelle social calls might be interpreted accordingly.

Pipistrellus kuhlii calls also last longer than those by *P. pipistrellus*, and an experienced listener might in many cases individuate this further difference by hearing the different 'rhythm' of the consecutive call components in time-expanded calls, which appears slower in *P. kuhlii* (Barataud, 1996). Duration, however, is one of the most variable parameters in Kuhl's pipistrelle social calls because it is related to the number of components, and may overlap between social calls of *P. kuhlii* and *P. pipistrellus*.

The parameter *fmax* differs clearly only between the Kuhl's pipistrelle and the 45 kHz *P. pipistrellus* phonic type. However, its measurement may not be reliable, as higher frequencies are more deeply affected by atmospheric attenuation (Griffin, 1971; Pye, 1980; Lawrence & Simmons, 1982).

A combined use of *fmin*, *freq*, and *dur* measured on time-expanded social calls should help to discriminate between field recordings of unknown *Pipistrellus* whenever the identification based on echolocation calls is uncertain. The differences in frequency values observed are not sufficiently large to permit the usage of heterodyne detectors for a social call-based species identification.

In areas where *P. nathusii* and *P. kuhlii* occur sympatrically, a discrimination based on social calls should be easy to carry out as the former species emits typical advertisment and social calls constituted by a main part with a larger (five to seven) number of components, and a final, higher-pitched trill (Barlow & Jones, 1996).

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Paracrobeles psammophilus sp. nov. (Nematoda: Cephalobidae) from El Saler, Valencia (Spain)

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Abstract

Paracrobeles psammophilus sp. nov. (Nematoda: Cephalobidae) is described from soil of the Dehesa de El Saler (province of Valencia, Spain). This is the second record of a species of the genus *Paracrobeles* Heyns, 1968. The new species is characterized by rounded cheilorhabdions and the large spicules and gubernaculum of males.

INTRODUCTION

The genus *Paracrobeles*, proposed by Heyns (1968) to include only *Paracrobeles laterellus* Heyns, 1968, a species recorded in South Africa, is characterized by the presence of a well developed median bulb with thick-walled lumen in the oesophagic metacorpus and by the absence of fringes in the labial probolae.

We have obtained nematodes from soil of the Dehesa de El Saler whose morphological features agree with those considered characteristic for the genus *Paracrobeles* Heyns,1968. However, characters such as rounded cheilorhabdions and the large size of spicules and gubernaculum in males permit a clear separation of the species.

MATERIALS AND METHODS

Sampling was conducted on a monthly basis for 12 consecutive months (April 1992–March 1993) in order to study the variations in nematode numbers throughout an annual cycle. The sample site was a small clearing in a pine *Pinus halepensis* forest located in the fixed and stabilized dunes of the Dehesa de El Saler (37°40′N, 0°30′E; UTM 30SYJ35). The vegetation in the clearing was typical Mediterranean underbrush (*Phillyreo angustifoliae–Rhamnetum angustifoliae*) (Costa & Mansanet, 1981).

Edaphic samples were collected with an 8 × 25 cm (inner diameter × height) metallic corer. Subsequently, the organic stratum (upper 7–8 cm) was separated from the mineral stratum consisting of the soil remaining within the corer. Using this methodology, 3 samples were collected every month throughout the study period from 3 different sampling sites in the study area. The samples were processed immediately. Samples of plant material were also taken at each sampling site to observe the presence of *Paracrobeles psammophilus* in the edaphic fraction associated with the roots. For each sample, we processed 100 cm^3 from each of the 2 edaphic strata by the Baermann funnel technique and 100 cm^3 by centrifugal flotation (Nombela & Bello, 1983), as well as 15 g of roots. Once isolated, the nematodes were fixed in a solution of 4% formaldehyde with calcium carbonate and mounted according to Seinhorst (1959, 1962).

Monthly variation in nematode numbers was calculated by taking into account only individuals obtained by the centrifugal flotation technique. This technique seemed more appropriate for quantitative analyses than

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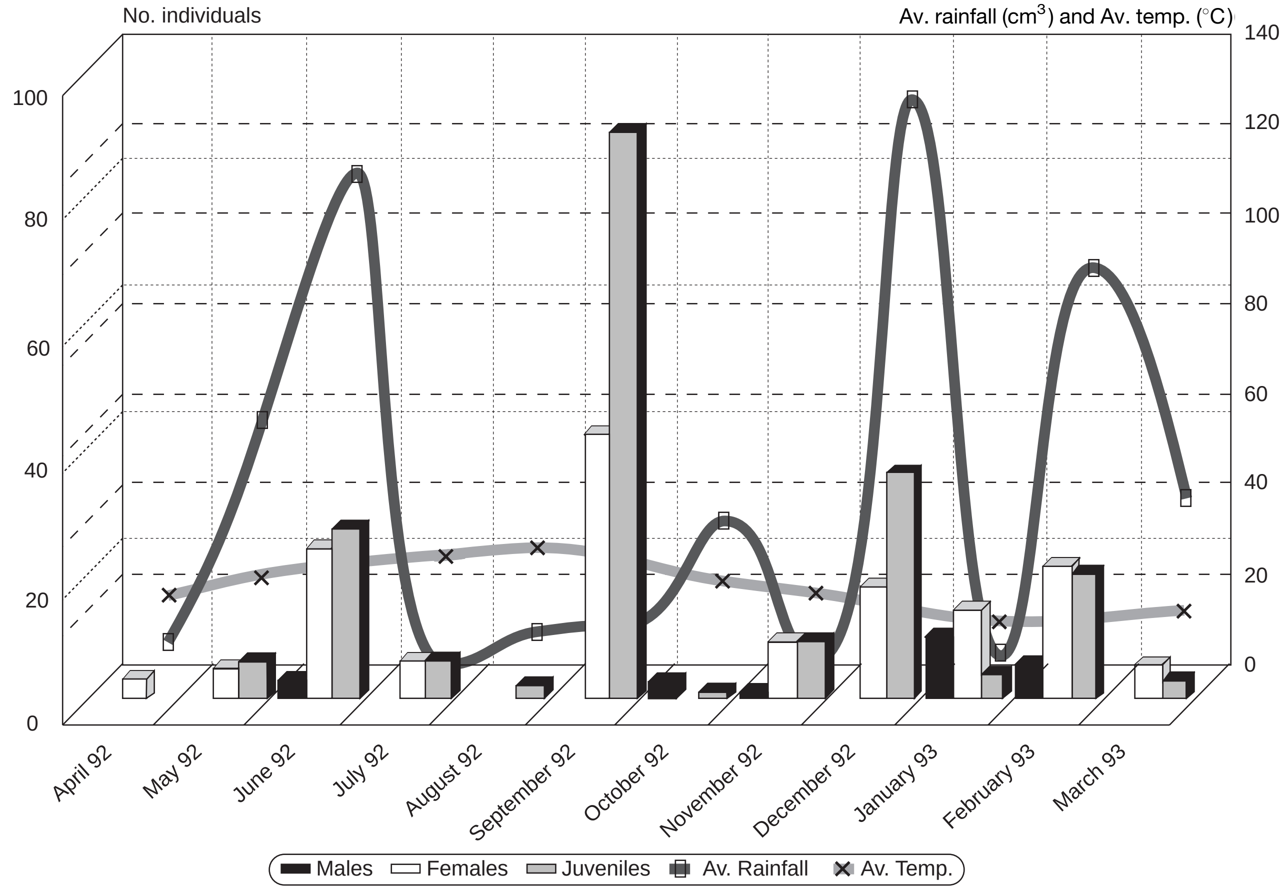

Fig. 1. Monthly variation in the number of individuals of *Paracrobeles psammophilus* sp.nov.

the Baermann funnel technique since it avoided problems associated with incubation or lack of mobility of the nematodes.

The frequency of *Paracrobeles psammophilus* was obtained using the formula Fr = N x 100/Nm and abundance was calculated as Ab = Ne x 100/Nt (Fr = frequency; N = number of samples containing *P. psammophilus*; Nm = total number of samples; Ab = abundance; Ne = number of *P. psammophilus*; Nt = total number of individuals in all the samples). Indices and abbreviations are taken from Southey (1978).

RESULTS

Our results indicate that *P. psammophilus* shows a preference for the mineral edaphic fraction of the soil in which we found a total of 349 individuals, 54 using the Baermann funnel and 295 using the centrifugal flotation technique. In the root soil fraction we found 62 individuals, and in the organic stratum 18 individuals, all obtained by centrifugal flotation.

Data on the monthly variation of this species are presented in Fig. 1, together with the mean monthly temperature and rainfall, kindly provided by the National Meteorological Center (Valencia). As can be observed from the figure, this species is relatively independent of temperature and of rainfall, and numbers peak in September, although they are present in the soil year round.

Paracrobeles psammophilus sp. nov. (Fig. 2)

Female (10 specimens measured, including the holotype). L = 495 ± 57 mm (mean ± SD) (range 360–552 mm); a = 13.28 ± 2.06 (10.00–16.65); b = 3.22 ± 0.25 (2.88–3.72); c = 10.00 ± 1.22 (7.06–11.23); c’ = 2.29 ± 0.24 (1.92–2.67); V = 61.23 ± 1.20% (58.89–63.41%).

Body almost straight, with length 495 57 (360–552) mm and maximum body width 38 ± 4 (32–42) mm, tapering toward both extremities. Cuticle coarsely annulated, with annuli 4 ± 0.4 (4–5) mm wide at midbody. First annules behind the head end divided into rectangular blocks by longitudinal lines. Lateral field marked by three crenate incisures.

Lip region continuous with body contour; cephalic width 15 ± 1 (14–16) mm. Labial probolae not fringed, deeply bifurcate 10 ± 0.6 (10–12) mm long. Cephalic probolae with pointed tips, unfringed, interspersed with sharply pointed tines. Amphid situated at the base of the lateral cephalic probola.

Cheilorhabdions rounded in optical section. Oesophagus 154 ± 14 (121–164) mm long. Stoma 13 ± 1

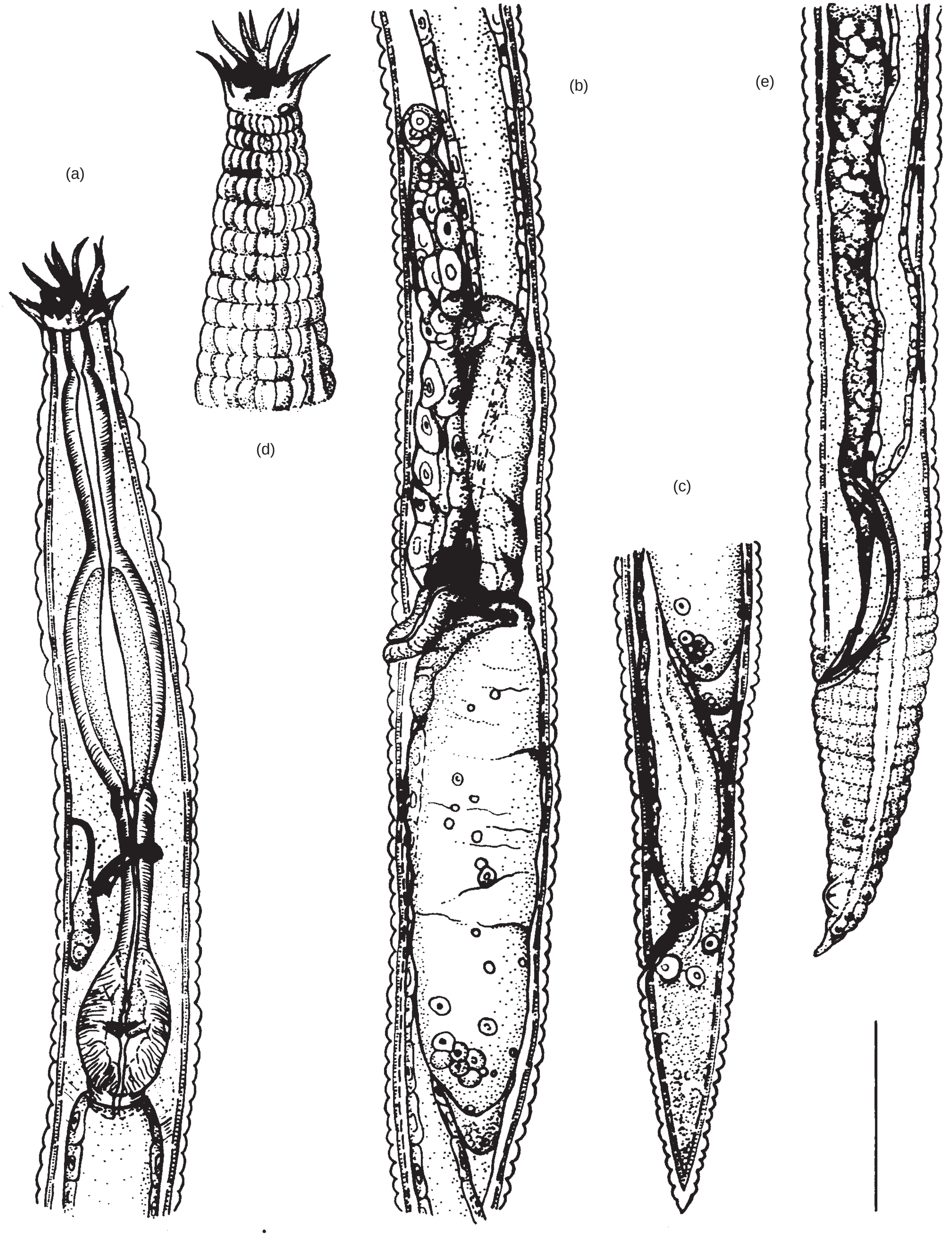

Fig. 2. *Paracrobeles psammophilus* sp. nov.: (a) oesophagic region; (b) female reproductive system; (c) tail region of female; (d) head region (surface view); (e) tail region of male. Scale bar = 40 μm.

(10–14) mm long, lightly sclerotized. Procorpus cylindrical, 36 ± 2 (32–40) mm long, widening to an elongate metacorpus with thick-walled lumen, which dilates to form a large triquetrous chamber 44 ± 3 (40–49) mm long, and 19 ± 2 (16–22) mm wide. Isthmus set off from median bulb by conspicuous transverse markings 35 ± 4 (30–40) mm long. Basal bulb 26 ± 2 (22–28) mm long, and 20 ± 1 (18–22) mm wide, with the usual valvular apparatus. Nerve ring encircling isthmus at 107 ± 11 (89–125) mm from the oral opening. Excretory pore 97 ± 16 (69–117) mm from the oral opening.

Female reproductive system cephaloboid. Ovary short; spermatheca at anterior flexure of ovary. Post-vulval uterine sac variable in length, 72 ± 16 (57–101) mm long.

Tail convex conoid, almost straight, 50 ± 2 (46–53) mm long and width of the body at anal level 22 ± 2 (18–24) mm. Rectum 20 ± 1 (18–22) mm long. Phasmid at 36 ± 5 (32–45) mm from posterior end of body.

Total body annuli 125 ± 4 (119–132), of which 35 ± 4 (28–43) occur from head to base of pharynx, 72 ± 3 (67–75) from head to vulva, 112 ± 4 (106–119) from head to anus, and 13 ± 1 (12–16) annuli between anus and end of nematode.

Males (10 specimens measured, including the allotype). L = 481 ± 40 (424–531) mm; a = 15.08 ± 2.38 (10.10–17.89); b = 3.31 ± 0.18 (3.05–3.60); c = 10.04 ± 1.09 (8.82–11.83); c' = 1.98 ± 0.25 (1.57–2.23); T = 48.26 ± 1.98 (45.57–52.11) %.

Similar to female in general appearance. Total length 481 ± 40 (424–531) mm and maximum body width 32 ± 4 (28–42) mm. Cephalic width 14 ± 1 (12–16) mm; labial probolae 11 ± 1 (9–13) mm long; oesophagus length 145 ± 6 (137–152) mm; stoma 12 ± 0.9 (10–14) mm long; procorpus length 34 ± 3 (28–38) mm; metacorpus 40 ± 2 (38–42) mm long and 19 ± 2 (16–22) mm wide. Isthmus 36 ± 2 (32–40) mm long; basal bulb 24 ± 1 (22–26) mm long 18 ± 2 (14–22) mm wide. Nerve ring at 106 ± 8 (87–113) mm from the oral opening. Excretory pore 93 ± 9 (75–107) mm from the oral opening.

Testis reflexed. Spicules arcuate, 51 ± 4 (42–57) mm long. Gubernaculum 25 ± 6 (20–39) mm long.

Tail convex conoid, slightly ventrally curved, 48 ± 4 (42–53) mm long. Width of the body at anal level 25 ± 2 (22–30) mm. Rectum 31 ± 9 (20–51) mm long. Phasmid at 27 ± 3 (21–34) mm from posterior end of body. One pair of lateral caudal papilla at the end of the lateral field; two pairs of lateroventral papillae, the first close to the tail terminus, and the second anterior to the first; one pair laterodorsal and one pair lateroventral close to the phasmid. One pair of lateroventral pre-anal papilla just anterior to the cloaca.

Total body annuli 125 ± 4 (119–131), of which 35 ± 3 (31–39) occur from head to base of pharynx, 112 ± 3 (106–116) from head to anus, and 14 ± 1 (11–15) annuli between anus and end of nematode.

Holotype: (female) L = 533 mm; a = 16.65; b = 3.29; c = 10.45; c' = 2.55; V = 60.98%. Specimen No. SA03PR-7, Nematological Collection of the Department of Animal Biology, University of Valencia (Spain).

Allotype: (male) L = 531 mm; a = 17.70; b = 3.54; c = 10.02; c' = 2.21; T = 45.57%. Specimen No. SA03CP-9, Nematological Collection of the Department of Animal Biology, University of Valencia (Spain).

Paratypes (nine females and nine males). Specimens Nos. SA01PR-4; SA08PR-1; SA10PR-6; SA12PR-6; SA12PR-7; SA12PR-10; SA03CP-5; SA03CP-7; SA03CP-10; SA10PR-8; SA10PR-9; SA06CP-5; SA07CP-2; SA11CP-4; SA11CP-4; SA03BP-7; SA03BP-8; SA07BP-5, Nematological Collection of the Department of Animal Biology, University of Valencia (Spain).

Type locality: Dehesa de El Saler (province of Valencia, Spain).

DISCUSSION

The new species agrees with the characteristics of the genus *Paracrobeles* (Heyns, 1968; Andrássy, 1984; Rashid, Heyns & Coomans, 1990). These include the structure of the oesophagus, of which the metacorpus is developed into an elongate median bulb with a large chamber, the labial probolae bifurcate, long, without fringes and the cephalic probolae sharply pointed, without fringes, and interspersed with sharp tines.

However, *P. laterellus* has a larger body size, proportionally shorter tail in females, and males have shorter spicules, and shorter gubernaculum. Finally, *P. psammophilus* shows rounded cheilorhabdions in optical section, not observed in *P. laterellus*. This combination of characteristics allow us to make a clear separation of both species (Table 1).

Paracrobeles laterellus Heyns, 1968, was described from three different localities in South Africa, east of Alldays in the northern Transvaal, and Orange River and the Kalahari Gemsbok Park, both in the northern Cape Province. The original description was based on the study of four female specimens and six male specimens. Rashid *et al.* (1990) found a new female specimen in Namibia (South West Africa). No other individuals of the genus *Paracrobeles* have been reported. In contrast, *P. psammophilus* seems to be a fairly abundant species (Table 2), although only at the Dehesa de El Saler, since it has not been found in other neighbouring areas with similar characteristics.

Acknowledgements

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Table 1. Morphometric characteristics of *Paracrobeles laterellus* according to various authors and of *Paracrobeles psammophilus* (all dimensions in lm). According to Southey (1978), L = length; a = body length/body width; b = body length/ oesophagus length; c = body length/tail length; c' = tail length/ body width at the anal level; V = length from vulva to anterior tip x 100/body length; T = length from cloaca to anteriormost part of testis 100/body length. Ranges and means (in parentheses) of each character are shown

	Paracrobeles laterellus			*Paracrobeles psammophilus*	
	Females		Males	Females	Males
Characteristic	Heyns (1968)	Rashid *et al.* (1990)	Heyns (1968)	This study	This study
L	530–740	570	570–660	360–552 (495)	424–531 (481)
a	17–20	17.9	17–25	10.0–16.7 (13.3)	10.1–17.9 (15.1)
b	3.3–4.1	3.7	3.5–3.9	2.9–3.7 (3.2)	3.1–3.6 (3.3)
c	11–15	11.4	11–12	7.1–11.2 (10.0)	8.8–11.8 (10.0)
c'		2.2		1.9–2.7 (2.3)	1.6–2.2 (2.0)
V	58–60	58.5		58.9–63.4 (61.2)	
Annuli	3	4		4–5 (4)	4–4 (4)
Cephalic width	14–17	13		14–16 (15)	12–16 (14)
Oesophagus		152		121–164 (154)	137–152 (145)
Stoma		11.5		10–14 (13)	10–14 (12)
Excretory pore-oral opening		106.5		69–117 (97)	75–107 (93)
Uterine sac		46		57–101 (72)	
Rectum		20		18–22 (20)	20–51 (31)
Phasmid-end tail	25–30	28		32–45 (36)	21–34 (27)
Tail length	50–60	50	50–55	46–53 (50)	42–53 (48)
Spicules			32–35		42–57 (51)
Gubernaculum			17–22		20–39 (25)
No. oesophageal annuli		48		28–43 (34.5)	31–39 (34.7)
No. oesophageal-vulva annuli		112		67–75 (71.8)	
No. vulva-anus annuli		180		106–119 (112.0)	106–116 (111.5)
No. tail annuli		18		12–16 (13.20)	11–15 (13.6)
Total body annuli		198		119–132 (125.2)	119–131 (125.1)

Table 2. Frequency and abundance of *Paracrobeles psammophilus* sp. nov. in the different edaphic fractions obtained by the centrifugal flotation technique. (Fr. = frequency; Ab. = abundance; org. = organic fraction; min. = mineral fraction; root. = root soil fraction)

	Fr. (org.)	Ab. (org.)	Fr. (min.)	Ab. (min.)	Fr. (root.)	Ab. (root.)
P. psammophilus	8.33	0.21	75.00	9.58	66.67	2.94

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A case of co-operative nursing and offspring care by mother and daughter feral horses

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INTRODUCTION

Among mammals, non-offspring nursing is the most extreme form of communal parenting. This is because lactation is the most energetically costly part of parental investment (Clutton-Brock, 1991; Packer, Lewis & Pusey, 1992). Non-offspring nursing is most common in species characterized by large litters and small kin groups (Packer *et al.*, 1992; e.g. lions *Panthera leo*: Pusey & Packer, 1994). Although non-offspring nursing has also been reported in monotocous species (e.g. water buffalo *Bubalus bubalus*, Murphey *et al.*, 1995; African elephant *Loxodonta africana*: Dublin, 1983; Lee, 1987; Indian elephant *Elaphus maximus*: MacKay, 1973; Rapaport & Haight, 1987; fallow deer *Cervus dama*: San José & Braza, 1993) it is almost always associated with reproductive errors (Riedman, 1982) such as milk theft or exclusive adoption (Packer *et al.*, 1992). However, simultaneous non-offspring nursing in monotocous species has been reported in some bat species (e.g. McCracken, 1984; Eales, Bullock & Slater, 1988), African elephants (Lee, 1987), and captive Indian elephants (Rapaport & Haight, 1987). Recent research, however, suggests that nutritive non-offspring nursing in African elephants is rarer than previously thought as most reported instances were probably non-lactating juveniles allowing infants to suckle (Lee & Moss, 1986; Lee, 1987, 1989).

In species in which co-operative nursing and offspring care is common, it is difficult to determine which factors facilitated its evolution. However, in species in which co-operative nursing and offspring care is rare we can examine the set of events and circumstances that cause it to occur in order to understand how it may have originated in species in which it is now common (Packer *et al.*, 1992).

Horses are a monotocous species (Platt, 1978) and non-offspring nursing is rare both in captive (Crowell-Davis, 1985) and feral populations (Tyler, 1972; Packer *et al.*, 1992). Mares are typically intolerant of non-offspring foals and of other mares that approach their young foals (Tyler, 1972; Feist & McCullough, 1976; Berger, 1986). Foals, however, may attempt to suck from mares that are not their mother (Crowell-Davis, 1985), even from juvenile or adult males (Tyler, 1972). The occasional instances of non-offspring nursing are characterised by short suckles and involve mistaken identity, milk theft or attempted milk theft (Tyler, 1972; Crowell-Davis, 1985; Berger, 1986). Adoption or swapping of offspring are rarer still and only observed in domestic horses where the mothers are confined and parturition is highly synchronized (Tyler, 1972; Crowell-Davis and Houpt, 1986; Huntingdon & Cleland, 1992), or it is induced by human intervention (Tyler, 1972). Thus previous instances of non-offspring nursing in horses are cases of reproductive error (Riedman, 1982). Shared care and nursing of an offspring by mares has not before been reported in equids.

OBSERVATIONS AND DISCUSSION

We have been studying the maternal behaviour of wild horses in the Kaimanawa ranges, New Zealand. Kaimanawa horses have been feral since the late 1800s and roam an area of between 600 and 700 km^2. The study population is described in more detail by Linklater *et al.* (in press). Kaimanawa horses live in year-round stable social groups called bands, containing from one to four adult stallions and one or more mares and their immature offspring. We have recorded over 3000 suckle bouts involving 113 foals from August 1994 until March 1997. Of these only one foal (F) has been observed to suck from more than one mare. F consistently nursed from two mares, who were themselves mother (M, born around 1986) and daughter (D, born 1993). It is unusual in feral horse populations for mothers and daughters to live together because daughters disperse around sexual maturity (Berger, 1986; Rutberg & Keiper, 1993; Monard, Duncan & Boy, 1996). However, occassionally a daughter will not disperse from her mother when the mother herself shifts into a new band before it is time for the daughter to disperse of her own accord. M and D joined their current band in late 1994 when D was a yearling still sucking from M. Consequently the stallion in the new band is not D's father, and M and D have remained in this band together.

Both M and D were judged pregnant from assays of oestrone sulphate concentration in faecal samples taken between March and September 1996 (Henderson *et al.*, 1997). However, parturition by M and D was not seen and they were observed with only one foal of about 3 days old between them.

To determine if the behaviour of M, D and F was

Table 1. Details of focal mares and foals

Symbol	ID	Name	Born[a]	Band size[b]	Experience	Foal born[c]	Obs[d]
D	021	Sassy	1993	8	Primiparous	2 Nov 96	1190
D1	009	Libby	1993	5	Primiparous	6 Nov 96	865
D2	169	Celeste	1993	3	Primiparous	1 Oct 96	1060
M	066	Banff	1986	8	Last foal 1994	2 Nov 96	1190
M1	127	Ulysses	1986	7	Last foal 1996	18 Oct 96	570
M2	100	Darcy	1987	7	Last foal 1993	22 Nov 96	710

[a] Date of birth estimated by tooth wear patterns during a muster in 1994 except for D2 who was known to be a yearling in 1994.
[b] No. of adult females in group.
[c] Accurate to ± 2 days.
[d] Total time that behaviour was sampled (min) from birth to 100 days of age.

different from those of normal mare–foal dyads, each mare was paired with two control mares. M and D were paired with mares who were of a similar age, parity and all had daughters. All six mares and their foals were sampled between six and 11 times from birth to 100 days of age in focal samples of 30–220 min. The total observation time is shown in Table 1.

F sucked alternately from M and D; 83% of suckles were followed by a suck from the other mare ($\chi^2 = 10.60$, $P < 0.01$). The total time F spent sucking per day (breaks in nipple contact between episodes of sucking within a bout were excluded), from M or D was less than the time control foals sucked from their mothers. Consequently, M and D each suckled F for less than half the time other mares suckled their foals, well outside the spread of times for all mares with female foals in 1996 (M = 19.36 min/day, D = 13.38 min/day; range for 20 other mares with female foals in 1996 = 39.73–146.09 min/day). For F, each sucking bout contained fewer breaks in nipple contact (F, 48% no breaks; controls, 25% no breaks), fewer sucking episodes were associated with butts (Fig. 1a), the proportion of unsuccessful sucks tended to be less (where an unsuccessful suckle is one lasting < 5 s, during which time milk is probably not transferred; Whittemore, 1980; Fig. 1b) and fewer sucking episodes were ended by the mother (Fig. 1c). F sucked far less than control foals but time spent sucking is an inaccurate measure of milk transfer (Cameron, 1998*a*, Cameron *et al.*, 1999). Fewer butts, terminations by the mother and breaks in nipple contact indicate lack of hunger or fewer breaks in the flow of milk (Lent, 1971; Gomendio, 1989). Furthermore, fewer unsuccessful bouts or bouts ended by mothers, indicate that F fed to satiation (Byers & Bekoff, 1990). These suggest that F received at least as much milk as other foals, and possibly more.

The mean distance between F and the mares M and D was greater than the mean distance between each of the control mares and their foals but the mean distance to the closest mother was similar (Fig. 2a). Similarly, the proportion of time that either of the mares and F spent within 2 body lengths (Fig. 2b), or were each other's nearest neighbour (Fig. 2c), was lower for M and D than for the other dyads, but similar when calculated as the proportion of time spent within 2 body lengths of, or nearest neighbour of, either mother.

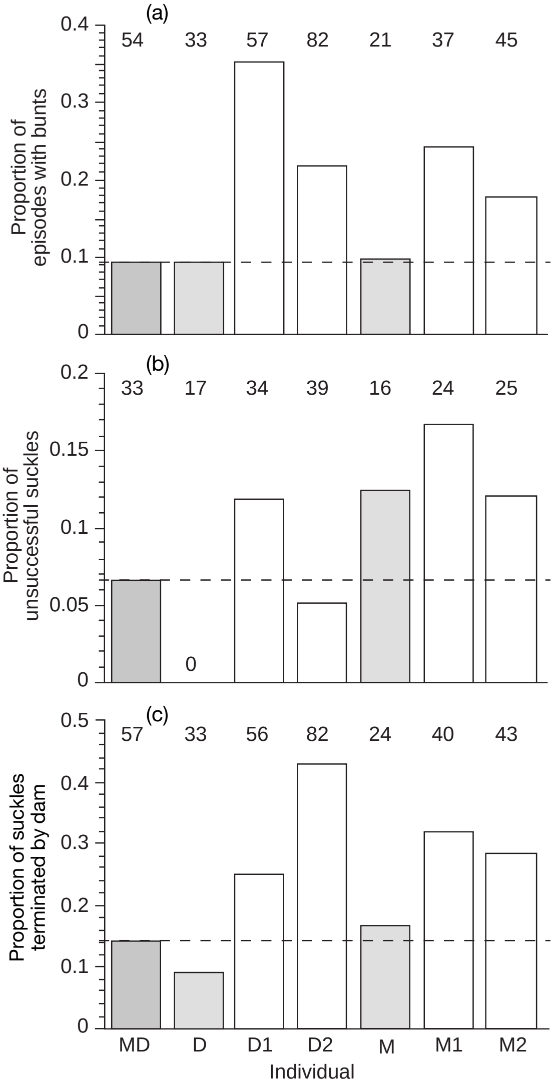

Fig. 1. Suckling behaviour of the shared foal compared to similar control foals that were raised singly from birth to 100 days of age. M refers to the elder mare who is mother of D, the younger mare and MD refers to the M and D combined (i.e. input from the foal's perspective), which is shown across the graph as a dashed line. Control mares, alike in all respects to either D or M except that they raised their foals singly, are designated D1, D2, M1 and M2. (a) The proportion of suck episodes during which the foal bunted, (b) the proportion of suck attempts that were unsuccessful, (c) the proportion of suckles that were terminated by the dam.

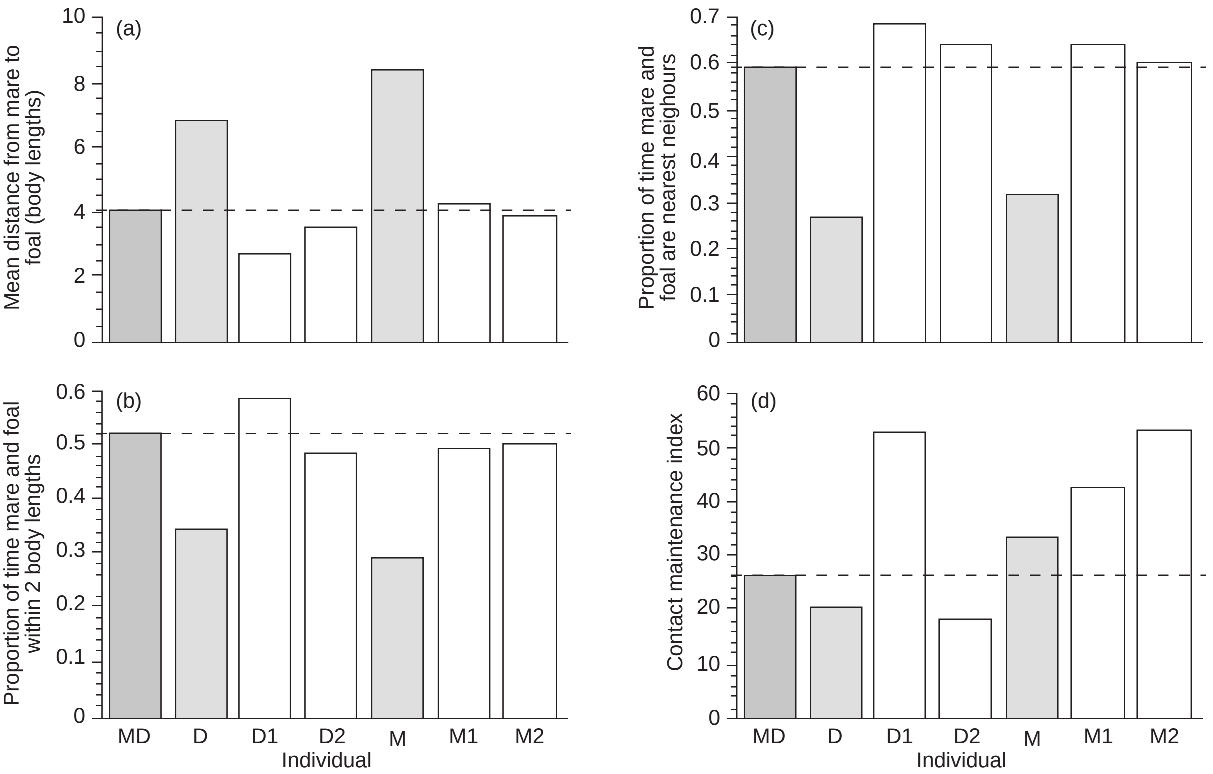

Fig. 2. Spatial relationship of the shared foal and the two mares M and D, compared to similar control foals and their mothers raised singly from birth to 100 days of age. M refers to the elder mare who is mother of D and MD refers to the combined scores for M and D, which is shown across the graph by a dashed line. Control mares, alike in all respects to either D or M except that they raised their foals singly are designated D1, D2, M1 and M2. (a) The mean distance between mare and foal, (b) the proportion of time that mare and foal were proximal (≤ 2 adult body lengths), (c) the proportion of time mare and foal were nearest neighbours, (d) contact maintenance index calculated as the difference between the percentage of approaches to within 2 body lengths and departures from within two body lengths that are due to the foal. Lower scores indicate more effort by the mares.

Consequently, each mare spent less time close to the foal, but the foal had at least one of the mares near as often as did singly reared foals. Moreover, each mare put similar effort into contact maintenance (difference in per cent approaches and per cent leaves between dyad that are due to the foal; Hinde & Atkinson, 1979) with F as did the control mares (Fig. 2d). Consequently, the differences in association patterns between F, M and D were solely a result of differences in F's behaviour; each mare still put a similar effort into maintaining contact with F as did control mares. The spatial patterns we describe suggest that the females are not only sharing nursing of F, but that they are sharing other aspects of foal rearing. The degree of sharing suggests that each mother contributes approximately half and the foal receives the same total input. This indicates a high level of co-ordination and co-operation between M and D.

Other foals were never observed to suck from mares that were not their mothers even when they became temporarily separated from their mother (3-12 hours, 5 foals). Furthermore, five foals orphaned during the study were never observed to successfully suck from any mare after the death of their mother. Two foals orphaned at a young age (6 and 21 days) attempted to suck from other mares but were prevented by aggressive responses from the mares. The youngest also attempted to suckle from its dead mother, another dead mare and an observer (EZC). Foals orphaned at an older age (3.5, 4 and 9 months) were never observed to attempt to suckle from any other horse, and all survived to adulthood. Most foal mortality occurs before peak lactation (50% before 20 days; Cameron, 1998*b*). The foals of eight mares died before peak lactation, but the mares did not let other foals of a similar age that were in their band to nurse from them.

In the unusual event that a mother and daughter feral horse lived in the same social group, co-operative suckling and offspring care occurred. Therefore, although horses are monotocous and usually live in non-kin groups they do have the capacity to co-operate in offspring care. Although this is the first documented case of cooperative offspring nursing and care in horses, we believe that it is probably not an isolated event. Shared offspring care and suckling have both been observed in managed semi-captive Icelandic horses (van Dierendonck, pers. comm.). Notably, despite numerous opportunities for shared suckling due to mare–foal separation or orphaning and foal death, shared suckling only occurred where mother and daughter lived in the same social group.

Previously reported studies of shared suckling in free living monotocous species have all involved closely related individuals. In Mexican free-tailed bats (McCracken, 1984) and in pipistrelles (Eales *et al.*, 1988) communal suckling is associated with relatedness. African elephants that have suckled non-offspring have been either sisters or grandmothers of the infant (Lee, 1989). In captive Indian elephants a grandmother not only suckled her grandson simultaneously with its mother but came into milk to do so. In addition, the grandmother would let no other calf suckle (Rapaport & Haight, 1987). Previous studies (e.g. Packer *et al.*, 1992) have suggested that co-operative nursing is most strongly associated with polytocous species but not monotocous species. The exceptions on both sides of this division suggest that it is not useful and it lacks explanatory power. The relatedness of mothers, however, seems to have an important role to play in the initial occurrence of co-operative offspring care. Consequently, kin selection is implicated in the evolution of co-operative nursing and offspring care.

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Morphology of spermathecae in the estuarine crab *Chasmagnathus granulata* Dana 1851 (Grapsidae, Sesarminae)

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Abstract

The morphology of the spermathecae (seminal receptacles) was studied in the grapsid crab *Chasmagnathus granulata*, both from the macroscopic and the microscopic points of view. Female adult crabs were collected throughout the year. Once in the laboratory, the right spermatheca was fixed in Bouin solution; histological sections were stained with haematoxilin–eosin. The observations indicated that the spermathecae belonged to the 'ventral type' described by Diesel (1991), related to the 'hard female' mating system of *C. granulata* described previously. They presented two distinct zones: the inner one (white-coloured), where the double layer oviduct opened ventrally; and the outer one, of translucent, 'jelly-like' appearance, where the communication with the genital duct could be observed. The spermatozoa were only observed in the 'jelly-like' zone, but not as discrete packets, suggesting that spermatozoa from different matings could mix in the spermathecae of *C. granulata*. The spermathecae also showed seasonal variation, with the lowest degree of development observed during autumn. Post-moulted females always showed collapsed spermathecae.

Key words: spermathecae, crabs, grapsids, *Chasmagnathus granulata*

INTRODUCTION

The spermatheca of the advanced Brachyura can be seen as an enlargement of the genital duct which extends from the ovary to the exterior and consists of four regions: (1) a short region connected to the ovary (oviduct), (2) an enlarged portion in which sperm is stored (spermatheca or seminal receptacle), (3) the part of the duct connected to the sternum (vagina), (4) the opening of the vagina to the exterior in the esternite of the sixth thoracic segment, usually called the vulva or genital opening (Hartnoll, 1968). The function of the seminal receptacle is the reception of the male sexual products during copulation and the subsequent storage until the fertilization of oocytes.

Spermathecae are paired sac-like organs, which present two zones: one dorsal, glandular zone of mesodermic origin, and the other one the ventral zone, with chitinized walls, of ectodermic origin (Diesel, 1991; Orensanz *et al.*, 1995). They are usually referred to as 'storage' and 'fertilization chamber', respectively (Diesel, 1990). The analysis of the stored sperm provides the means for measuring the occurrence of copulation; in this sense, spermathecae can be studied to determine the environmental impact of male-only fisheries, as reported for several crustacean species (Saint-Marie & Lovrich, 1994; Gardner, Rush & Belilacqua, 1998).

Chasmagnathus granulata (Brachyura, Grapsidae) is a semi-terrestrial estuarine crab, inhabiting the South American Atlantic coast from Río de Janeiro, Brazil (22°S), to San Matías Gulf, Argentina (41°S) (Boschi, 1964). This species forms dense populations in salt-marsh environments, such as dos Patos Lagoon, Mar Chiquita Lagoon and Samborombón Bay. During the reproductive period reported for *C. granulata* living in Samborombón Bay, i.e. September to March (spring–summer) (Rodríguez, 1991), ovigerous females are likely to migrate to estuarine waters of high salinity and low temperature, suitable for hatching and larval development. After four zoea instars (Boschi, Scelo & Goldstein, 1967), larvae return to the coast as megalopa, to moult to the first juvenile instar. Juvenile crabs reach the adult condition after several moults. Several reproductive features of this crab have been recently studied (Stella, Lopez & Rodríguez, 1996; López, 1997; López, Stella & Rodríguez, 1997; López Greco & Rodríguez, in press), as well as ecological aspects of its reproduction (Ruffino, Telles & D'Incao, 1994). Moreover, the effects of pollutants on reproductive events of *C. granulata* have also been examined (Rodríguez & Medesani, 1994; Rodríguez, Schuldt & Romano, 1994).

This work was aimed at characterizing the changes in the morphology of spermathecae throughout the year, considering both moulting and reproductive events, in *C. granulata*.

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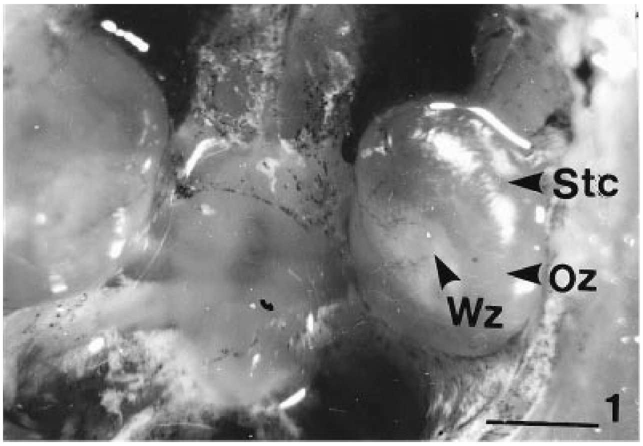

Fig. 1. Spermathecae (Stc) of *C. granulata*, in a general dorsal view (carapace has been dissected). Wz, white zone; Oz, opalescent zone. Scale bar = 0.5 mm.

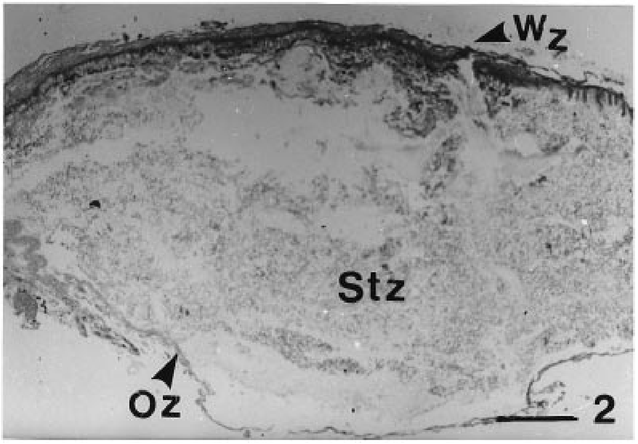

Fig. 2. Spermatheca of *C. granulata* (histological section). Wz, white zone; Oz, opalescent zone; Stz, spermatozoa. Scale bar = 0.1 mm.

MATERIALS AND METHODS

Female crabs were collected in Samborombón Bay (36°18′S, 56°47′W), Argentina, throughout the year. Once in the laboratory, maximum carapace width (CW, behind the third cephalothoracic spine) was measured using a vernier calliper (precision: 0.02 mm). All females were > 22.7 mm CW, the size at which the onset of sexual maturity occurs (López, 1997; López *et al.*, 1997).

Moult stages, as well as ovarian maturity stages, were determined according to previous works on the same species (Rodríguez *et al.*, 1994; López, 1997; López Greco & Rodríguez, 1999).

For the macroscopical description of spermathecae, at least 20 females were sacrificed monthly. These females were also used for determining gonadal and hepatopancreatic indexes (López Greco & Rodríguez, in press).

For the histological analysis, 15 ovigerous females collected during the reproductive period of the species (isolated from males) were maintained until larval hatching. These females were cold-anaesthetized at −20 °C during 15 min, to dissect the right spermatheca. These spermathecae were fixed in aqueous Bouin solution for 1.5 h, dehydrated, embedded in paraffin and cut in sections of 5–7 μm thickness. Finally, histological sections were stained with haematoxilin–eosin (according to the methodology described by López *et al.*, 1997) and observed under a light microscope.

RESULTS AND CONCLUSIONS

The spermathecae of *C. granulata* were observed as a pair of globular, sac-like structures of approx. 5–6 mm (diameter), without any difference between the right and the left spermatheca of the pair (Fig. 1). The communication with the oviduct was similar to that described for *Sesarma bidentatum* (Hartnoll, 1964) and *Aratus pisonii* (Hartnoll, 1965).

We recognized two zones in the spermatheca: the first, a white-coloured zone in an inner position (sagittal plane), comprised the opening of the short oviduct; the second, had a more conspicuous, opalescent and 'jelly-like' consistence, connecting to the short cuticular genital duct, which was approx. 1–2 mm long. The spermathecae of *C. granulata* belong to the ventral type described by Diesel (1991) (Fig. 1) and could be related to the 'hard female' mating system, previously described for *C. granulata* (López & Rodríguez, 1997). According to this pattern (ventral type spermatheca-hard mating system), *C. granulata* seems to present the same general trend as the Calappidae, Geryonidae, Leucosiidae, Parthenopidae, Parathelphusidae, Corystidae, Ocypodidae and Majidae (Diesel, 1991). No velum or any analogous structure was seen partitioning the spermatheca, as is the case in *Cancer gracilis* (Orensanz *et al.*, 1995).

We have seen changes in size and consistence of spermathecae during the year. During the winter, spring and summer they were globular conspicuous structures (Fig. 1) with their two zones clearly distinguished, while during the autumn they were collapsed, and they even seemed absent (the greater collapse was observed in May). No differences between spermathecae from females of different stages of ovarian maturation were detected.

In relation to the moult cycle, both zones of spermathecae could be distinguished only during the intermoult and premoult periods; the maximum collapse was seen in recent post-moulted females. When they were collapsed, only the opalescent zone was distinguishable (although it was seen as transparent). These observations were similar to those of Ryan (1967) in *Portunus sanguinolentus*; in which the ovary and the seminal receptacle underwent cyclic changes; the ovary in a coordinate way with the moult cycle.

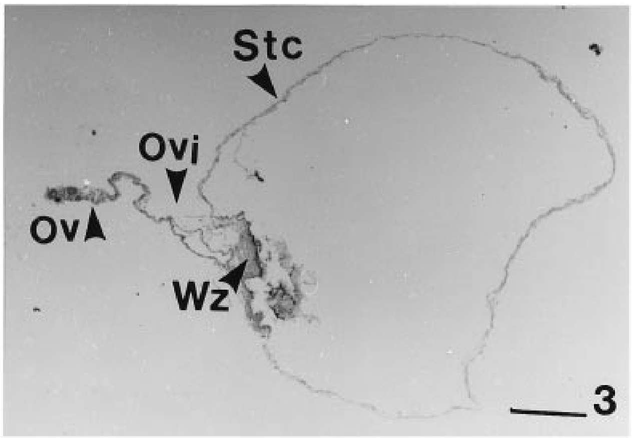

Fig. 3. Relationship between ovary, oviduct and spermatheca. Ov, ovary; Ovi, oviduct; Stc, spermatheca; Wz, white zone of the spermatheca. Scale bar = 0.1 mm.

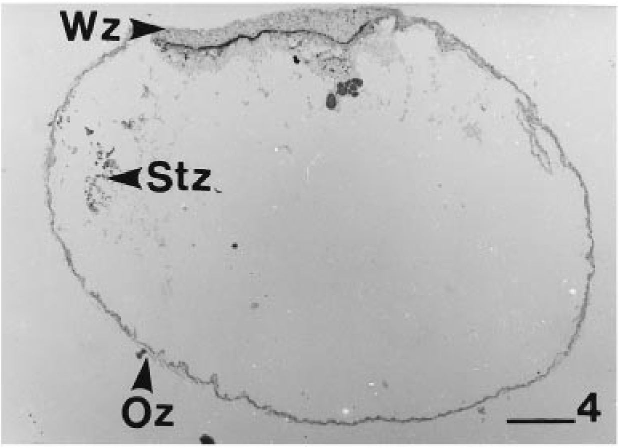

Fig. 4. Spermatozoa in the spermathecae of *C. granulata*. Stz, spermatozoa; Wz, white zone; Oz, opalescent zone of the spermathecae. Scale bar = 0.1 mm.

In histological sections, the spermatozoa were only observed in the more ventral zone (whose wall was seen as a double cellular layer, Figs 2–4). A high number of spermatozoa was observed in spermathecae of post-spawned females isolated from males (Fig. 2). Therefore, after each mating, females of the studied species may accumulate enough spermatozoa in their seminal receptacles to fertilize at least one batch of eggs in the male's absence. This sperm storage may thus allow females to maximize the probability of successful broods after insemination (Beningher, Lanteigne & Elner, 1993). Although many crab species fertilize several clutches of eggs from a single mating (e.g. Hartnoll, 1965; Diesel, 1989, 1991; Fukui, 1990); *C. bairdii*, for instance, can only fertilize one clutch of eggs from one insemination (Paul & Paul, 1992).

No clear arrangement of spermatozoa in different 'discrete' areas or spermatozoa 'packages' was observed (Figs 2–4), as described for *Portunus sanguinolentus* (Ryan, 1967), *Inachus phalangium* (Diesel, 1988, 1991) and *Pseudocarcinus gigas* (Gardner *et al*., 1998). Therefore, *C. granulata* could present a mix of spermatozoa from different matings, as other Grapsidae like *Metopaulias depressus* (R. Diesel, pers. comm.) and *Sesarma reticulatum* (Zimmerman & Felder, 1991).

The anatomical relationship among several parts of the genital duct of *C. granulata* is shown in Fig 3, particularly for the sequence ovary–oviduct–spermatheca. The photographed female presented a few spermatozoa (< 50); it was an ovigerous female that moulted after larval hatching, and was maintained isolated from males. Although a few spermatozoa were present in this case, we have never seen ovigerous females that have had a clutch of eggs, then moulted and have not mated. The absence of trans-moult spermatophore retention was also observed in *Aratus pisonii* (Hartnoll, 1965), another crab having the ventral type-hard mating system described by Diesel (1991).

The presence of spermatozoa in the spermatheca does not necessarily imply that they are viable, as in *C. opilio* (Paul, 1984). On the other hand, a minimum number of spermatozoa is necessary to allow spawning in the same species (Saint-Marie & Lovrich, 1994). According to our results, these kind of studies would be relevant in *C. granulata*, as well as the confirmation of the trans-moult absence of spermatophore retention.

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