





## ORIGINAL RESEARCH

**Behavioural repertoire and the effect of male removal in a geotrupid beetle with parental care**J. Kiss<sup>1,2</sup> , M. E. Rosa<sup>3</sup> , R. Rácz<sup>2,4</sup>, A. Kosztolányi<sup>3</sup>  & Z. Barta<sup>1</sup> <sup>1</sup>ELKH-DE Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, Debrecen, Hungary<sup>2</sup>Juhász-Nagy Pál Doctoral School, University of Debrecen, Debrecen, Hungary<sup>3</sup>Department of Ecology, University of Veterinary Medicine Budapest, Budapest, Hungary<sup>4</sup>Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary**Keywords**

beetles; cooperation; nesting behaviour; male removal; parental care; sex roles; *Lethrus apterus*; parental behaviour.

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**Abstract**

Although biparental care is thought to be rare among insects, it has evolved independently numerous times resulting in diverse forms and varying levels of parental involvement. Several theoretical studies address potential factors promoting biparental care in beetles; however, empirical studies target principally only species of Scarabaeidae and Silphidae families. Here, we investigated the behavioural repertoire of the breeding pairs in two natural populations of a geotrupid beetle *Lethrus apterus* with parental care. Changes in female care as a response to the male's absence were also investigated by experimentally removing males from pairs. We found that males spend the majority of their time at the nest entrance, while females leave their nest and collect leaves at a significantly higher rate than males. We also showed that males often desert their nests and were usually replaced by new males in a short time. The desertion of males, however, does not seem to influence females' behaviour. These findings indicate that *L. apterus* may have a dynamic mating system with typically short-term mate fidelity. Furthermore, our results have advanced our understanding of parental roles and nesting behaviour and contributed to the description of the distinct roles of parents in different phases of nesting events in *L. apterus* beetles.

**Introduction**

Parental care, defined as any behaviour of the parent that increases its offspring's fitness (Trumbo, 2012), is ubiquitous in the animal kingdom, yet the rich diversity of this behaviour exhibited by insects awaits to be fully explored and understood (Costa, 2006; Cunningham, 2020). As in vertebrates, insect females provide care more frequently than males in cases where care is part of the behavioural repertoire of the species (Kokko & Jennions, 2012; Trumbo, 2012). When male care is present, it is usually performed together with a female resulting in biparental care (Gilbert & Manica, 2015).

An important concept of parental care is that, although the benefits of care are shared between the parents, individuals need to pay the costs of their own parental effort alone resulting in a conflict of interest between the parents over the amount of care (Parker et al., 2002). Due to this conflict, each parent is expected to seek to reduce their personal costs by delegating as many parental duties as possible to their partner. Moreover, by being the first to leave the brood after mating, individuals could not only eliminate all costs of care but gain extra mating opportunities (Webb et al., 1999). Desertion,

however, can mainly be beneficial if the chance of finding a new mate is high (Kokko & Jennions, 2008).

The existence of biparental associations implies that in some cases, males can benefit from staying with the family. These benefits may include increased offspring survival (Hunt & Simmons, 2002a), more ensured paternity by keeping rival males away (Kvarnemo, 2006; McNamara & Wolf, 2015; Remeš et al., 2015) and/or by enhanced attractiveness meaning more future mating opportunities (Chemnitz et al., 2017; Keppner & Steiger, 2021).

Even in biparental relationships, individuals often show different levels of parental investment, and parents may respond in different ways to the decrease in their partner's effort (Harrison et al., 2009). In biparental care system, when a partner decides to desert or becomes handicapped or dies, we may expect that its mate will, to some extent, increase its parental investment (McNamara et al., 2003). The overall level of care, however, will likely be lower than it would be if the two parents contribute fully (Houston et al., 2005; McNamara et al., 2003), potentially resulting in reduced offspring fitness (Musa, 2012; Royle et al., 2002). On the other hand, if the parent was already working at its near-maximum capacity, no

increased investment may be possible. In this case, no change in parental behaviour can be expected after mate desertion/disappearance (Smiseth & Moore, 2004).

A common way to investigate how flexible parental care decisions are, and how those affect reproductive success, is to experimentally remove one of the parents. Burying beetle species are frequent subjects of this kind of experiment (*Nicrophorus spp.*, see for example, Pilakouta et al., 2015; Smiseth et al., 2005; Smiseth & Moore, 2004; Suzuki & Nagano, 2009). In this group, parental care is flexible, and both males and females can perform the same parental behaviour within a uni- or biparental system (Eggert & Müller, 1997; Müller et al., 2007; Paquet et al., 2017; Scott, 1998). Interestingly, results about females' willingness to modify their parental investment after the removal of their mate are contradictory (Fetherston et al., 1994, but see also Rauter & Moore, 2004; Smiseth et al., 2005). Like silphid beetles, several species of the Scarabaeidae and Geotrupidae families are also known to show biparental care (Lukas, 2013), but the parental roles and efforts, especially in the latter group, are less explored. To identify the factors that favour the evolution of biparental care in beetles and those that promote the subsequent diversification and maintenance of care, empirical studies on diverse beetle taxa show parental care is needed.

*Lethrus apterus* (Coleoptera: Geotrupidae) is an iteroparous, flightless burrow-digging beetle with predominant female care (Kosztolányi et al., 2015). Unlike other species of Geotrupidae, in *L. apterus* (and in the other members of Lethrinae subfamily), the adults and larvae feed on only plant materials (Nikolajev, 2003). The sexes are dimorphic as only males have ventral mandibular processes, also called tusks. Tusks serve as weapons in intrasexual contests for females (Nikolajev, 2003; Rosa et al., 2018). Adults usually emerge in March and start looking for mates shortly afterwards. Typically, the male searches and visits tunnels occupied by females (Frantsevich et al., 1977). The density of the tunnels can reach up to 10–15 burrows per m<sup>2</sup> (Frantsevich et al., 1977; Nikolajev, 2003). Since mating takes place in the tunnel (Frantsevich et al., 1977; Nikolajev, 2003), our knowledge about the mating behaviour of *L. apterus* is limited. After pair formation, the tunnel functions as a nest which is excavated, expanded and maintained through the breeding season by the owners. In a 60–100 cm deep nest, usually 5–8 *L. apterus* larvae develop separately in their brood chambers (pers. obs., Frantsevich et al., 1977; Kosztolányi et al., 2015; Nikolajev, 2003). According to recent studies about the parental care of *L. apterus*, while females mainly work on provisioning each brood chamber with plant materials, the role of males may be more restricted to the defence of the nest against conspecific intruder males as well as predators or parasites (Kiss et al., 2020; Kosztolányi et al., 2015; Nikolajev, 2003; Rosa et al., 2017). However, the exact composition of male and female behavioural repertoires and time budgets during care are less known. In addition, the results of the recent investigations are inconsistent with earlier literature. *L. apterus* beetles were considered to be monogamous (Hamilton, 1979). Earlier literature also describes that, similarly to *Cephalodesmus* dung beetles with biparental care (Dalglish & Elgar, 2005; Monteith

& Storey, 1981), the male collects and transports leaves into the nest, while the female processes the collected plants and forms balls for the larvae inside the nest (e.g. Emich, 1884; Schreiner, 1906; but see also Kosztolányi et al., 2015). Moreover, it was considered that after forming the leaf balls, the female lays one egg into each leaf ball and then rolls them to the end of the brood chambers (Emich, 1884). However, our experience of nest excavation disproves this, as in all cases we found the egg behind the leaf ball, separated by a thin wall of soil (pers. obs.). These ambivalent characterisations justify a re-examination of the parental care system in *L. apterus*, including the clarification of the roles of the sexes.

In this study, our first aim was to describe the above-ground activities of the sexes in two natural populations of *L. apterus* beetles and thereby clarify the roles of *L. apterus* males during brood care. We video-recorded above-ground behaviour, like leaf hoarding, nest guarding, and nest construction or maintenance of the observed individuals around the nests. If the main role of the males is guarding, then we would expect that their range of movement is restricted to the entrance and close vicinity of their nest, while females would exhibit more diverse behaviour. Our second aim was to test by experimental male removal whether females modify their parental behaviour as a response to the disappearance of their partner. Finally, we investigated the effect of male removal on reproductive success.

## Materials and methods

### Fieldwork procedures

Our investigation was carried out over 2 years at two field sites. The first field study was performed between 27 April and 22 May in 2017 near Debrecen, Hungary (47°31'28.6" N 21°34'30.4" E) on a grassland patch of cc. 1900 m<sup>2</sup>. Firstly, we caught females moving on the surface and marked their elytra and pronotum uniquely with a paint marker (Uni Paint, Oil Base Paint Marker PX-20 Medium, Japan or Edding 780 gloss paint marker). After that, if a marked female was observed to collect leaves at least twice and carried those to the same nest, we tagged the nest with a flag, lured the resident male out from the nest with a blade of grass and gently marked its head with a paint marker. We used this method to mark males to avoid desertion as males are more sensitive to handling (JK and MER personal observation). After that, we started recording the activities of the beetles for approximately 4 h (depending on weather conditions; range of 3.47–4.83 h) using an action camera (SJCAM SJ4000 HD Action Cam, 1080P) mounted on a tripod approximately 20 cm from the entrance of the nest. On the following day, we randomly assigned the previously marked nests to either control or treatment group. In the treatment group, we removed the male from the nest and released it 32 ± 1 m (mean ± SD) away from the nest. No male found the way back to the treated nest during our investigation. Males were not removed from the control group, only lured out from the nests to control for the disturbance around the burrow. Immediately after the treatment, we recorded the activities at the nests for another 4 h (also depending on

weather conditions; range of 3.73–4.83 h). The experiment was completed at 35 nests (18 control and 17 treated nests).

The second study was carried out between 28 April and 14 May 2018 at Ózd-Susa, Hungary (48°16'32.8" N 20°14'46.3" E). We used the same protocol as above, except that removed males were released further (250 ± 20 m because of the vegetation pattern and topography) and the above-ground activities at the nests were recorded not on the second but on the third day (range of 4.14–4.84 h in case of the pre-treatment videos, and range of 2.72–4.34 h in case of the post-treatment ones). We completed the experiment at 22 (12 control and 10 treated) nests.

At the end of the breeding season, we marked all investigated nests in the Ózd-Susa population with a flag to ease their later localization. Between 23 May and 15 June, we excavated all the marked nests to determine their reproductive success. We recorded the number of offspring as well as the number of empty/unsuccessful brood chambers (i.e. the chambers filled with plant material but contained no living larvae).

In both populations (Debrecen and Ózd-Susa), the resident male was often replaced by a new, unmarked male even during the pre-treatment observations, and new males were often found in the place of the experimentally removed males as well. Furthermore, in some control nests, where we did not expect any partner changes, resident males deserted by the end of the experiment (see Table 1).

## Data analyses

Video footages were analysed by the Mwrap event recorder software (Bán et al., 2017). We coded the following events: duration of being away from the vicinity of the nest (i.e. staying outside of a 10 cm radius circle), the number of leaf collecting events (i.e. food provisioning), duration of staying in the vicinity of the nest (i.e. within a circular area with a radius of 10 cm around the nest entrance), duration of being at the entrance of the nest (considered as nest guarding) and the frequency of removing soil from the nest (indicating nest construction or maintenance). All data and statistical analyses

were performed in the R statistical environment (version 4.2.2, R Core Team, 2022).

On account of differences in the schedule between the studies, we analysed the data separately for the two populations as well as for the pre- and post-treatment conditions.

## Frequency and duration of activities

To investigate the parental roles of the sexes, we used the behavioural data collected only from the pre-treatment (first day) recordings.

We fitted generalized linear mixed effect models (hereafter GLMM(s)), specifically Hurdle negative binomial GLMMs (Panaccio et al., 2021; Zhang, 2019) by using the glmmTMB package (Brooks et al., 2017) to analyse the counted activities, because of their skewed distribution. Hurdle negative binomial models are proposed to handle overdispersion and deal with zero inflation without discriminating between the zeros' origins (i.e. zeros derived due to the absence or no-detection; Zeileis et al., 2008; Zuur et al., 2009). We used the count data of the following activities for each identified beetle per footage: (i) leaving the vicinity of the nest, (ii) being in the vicinity of the nest, (iii) emerging at the entrance of the nest (coming up from the nest), (iv) leaf collection and (v) removing soil from the nest (i.e. nest maintenance). The natural logarithm of the length of the videos (in hours) was included as offsets into the models, which practically means that we compared rates of events instead of their raw count numbers. We used 'sex' as an explanatory variable, and date of recordings and nest ID as random factors in the models.

To analyse differences in the mean duration of the activities, we fitted linear mixed effect models (hereafter LMMs) by using 'glmmTMB' function with gaussian distribution. We calculated the mean duration (in sec) of time spent (i) away from the nest, (ii) in the vicinity of the nest and (iii) at the entrance of the nest (i.e. guarding). We log-transformed these duration values and used them as response variables. Here, 'sex' as an explanatory variable and date of recordings and nest ID as random factors were also used in the models.

**Table 1** The numbers of the *Lethrus apterus* nests where previously marked resident males, unmarked new males, and no males were found during the pre-treatment (first day in both populations) and post-treatment part (second day in Debrecen and third day in Ózd-Susa population) of the experiments from Debrecen (a) and Ózd-Susa (b). In the last lines, the total number of control and manipulated nests are given

Number of nests	Control nests		Manipulated nests	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
<b>(a) Debrecen</b>				
With resident males	14	8	15	-
With no males	0	0	0	3
With new males	4	10	2	14
Total number of nests	18		17	
<b>(b) Ózd-Susa</b>				
With resident Males	9	0	9	-
With no males	0	5	0	3
With new males	3	7	1	7
Total number of nests	12		10	

**Table 2** Pre-treatment sex differences in rate (occurrence/h) and mean duration (in seconds) of activities related to parental care in two populations of *Lethrus apterus* in Debrecen (2017) and Ózd-Susa (2018)

Activity trait	Debrecen		Ózd-Susa	
	$\chi^2$	P-value	$\chi^2$	P-value
Rate of leaving the vicinity of the nest	132.020	<0.001	49.708	<0.001
Rate of being in the vicinity of the nest	22.767	<0.001	4.6882	0.096
Rate of emerging at the entrance of the nest	27.936	<0.001	10.75	0.005
Rate of leaf collection	128.64	<0.001	48.798	<0.001
Rate of nest maintenance	11.468	<0.001	9.638	0.008
Mean duration of being away from the vicinity of the nest	45.939	<0.001	28.995	<0.001
Mean duration of staying in the vicinity of the nest	16.418	<0.001	9.239	0.002
Mean duration of sitting at the entrance of the nest	40.910	<0.001	22.197	<0.001

Results of the likelihood ratio tests are shown (d.f. = 1, for all cases). Rate variables were investigated using Hurdle negative binomial GLMMs, and duration variables using LMMs. Significant results are highlighted in bold.

## Experimental manipulation of parental care

Because male changes occurred also at unmanipulated nests (see Results), instead of comparing the two treatment groups (control vs. manipulated), we used a ‘male status’ variable, indicating whether a marked resident male, a new, unmarked male, or no male was present beside the female.

To determine the effect of male absence on females’ behaviour, we used the counts and the durations (s) of activities (see above) from the post-treatment recordings of females as response variables.

To analyse the rate of the activities, we used negative binomial GLMMs with log(time) offset since there was no excess of zero values in the data of females. For explaining differences in the mean duration of the activities, we used LMMs. In all models, we used ‘male status’ (marked resident male, new unmarked male, or no male, see above) as a factor, and the date of recordings as a random factor.

## Reproductive success

Offspring number and the number of empty/unsuccessful brood chambers were analysed using GLMMs with negative binomial distribution. We used the factor ‘male status’ (i.e. new unmarked male or no male as the original male was not present at any of the control nests, see Table 1) as an explanatory variable in both models.

The significance of the explanatory variables in each model was tested with a likelihood ratio test using the ‘drop1’ function in R. To illustrate the effects of the factors on figures, we extracted estimated marginal mean (EMM) effects, along with associated standard errors (SE), using the ‘emmeans’ function of the emmeans package (version 1.8.4., Lenth, 2023). Log-transformed durations were back-transformed to their original scales before plotting.

## Results

### Occurrence of male desertion

We found that males routinely deserted the nests in both populations, leaving females and the brood behind, regardless of the

treatment (Table 1). Indeed, in the Ózd-Susa population, no resident male was present in any of the studied nests by the third day.

### Parental care behaviours

During the pre-treatment days in both populations, females left the vicinity of the nest and collected leaves at a significantly higher rate, and this resulted in spending more time out of the vicinity of the nest than males (Table 2, Figs 1a,d and 2a). Meanwhile, males spent significantly more time in the vicinity and at the entrance of the nests than females in both populations (Table 2, Fig. 2b,c). In addition, females emerged at the nest entrance at a significantly higher rate, and they removed soil from the nest (i.e. nest maintenance) more frequently than males (Table 2, Fig. 1c,e).

### Male removal

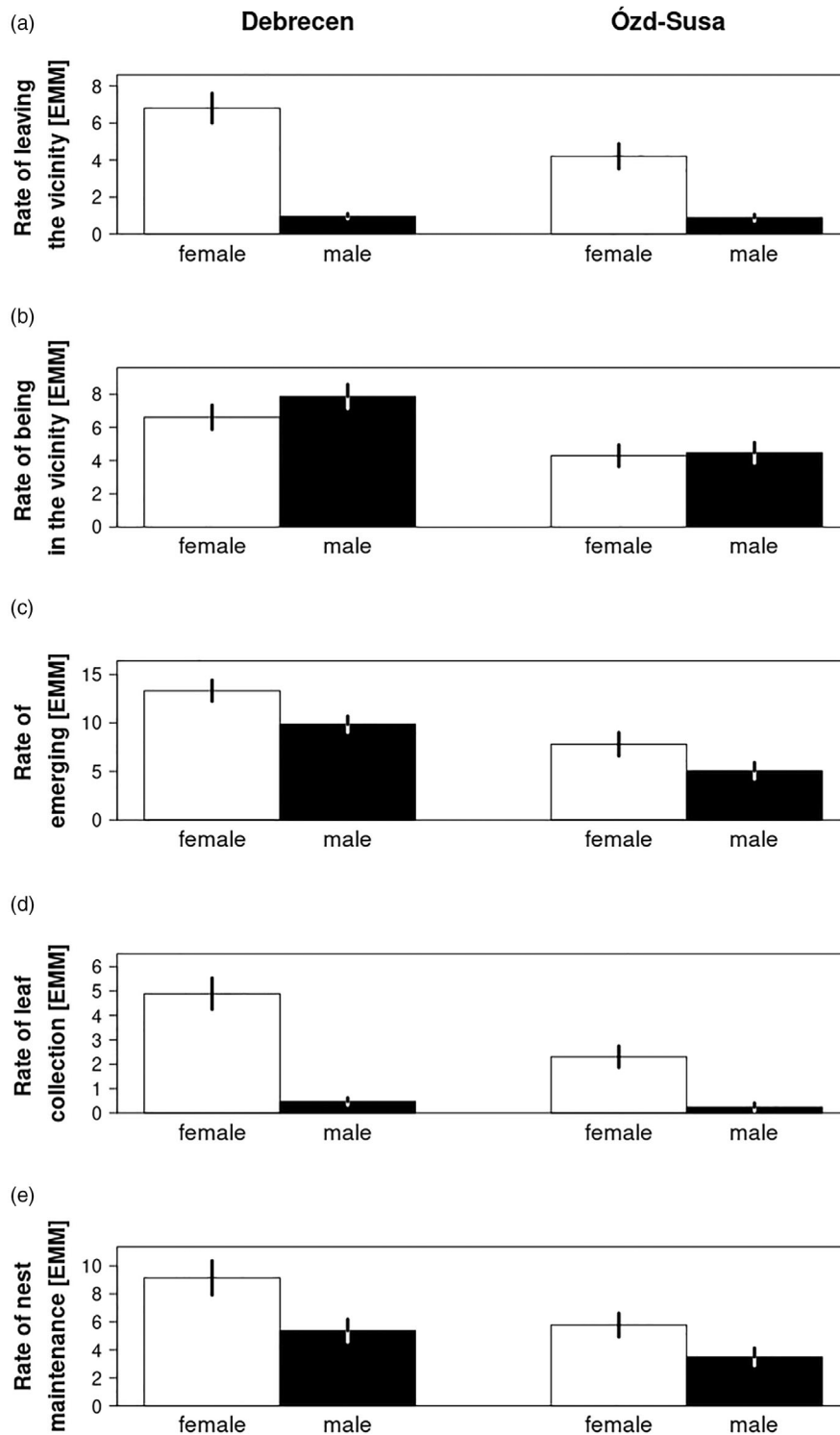
‘Male status’ (i.e. marked resident or unmarked new or no male) did not affect the females’ parental behaviours overall (Table 3). However, we found that ‘male status’ affected the nest maintenance rate of females in Debrecen (Table 3). Specifically, females paired with new unmarked males pushed out soil from the nests more frequently than females with the original, marked resident or without a male (Fig. 3). We found no difference related to the ‘male status’ of the females in the Ózd-Susa population (Table 3).

### Reproductive success

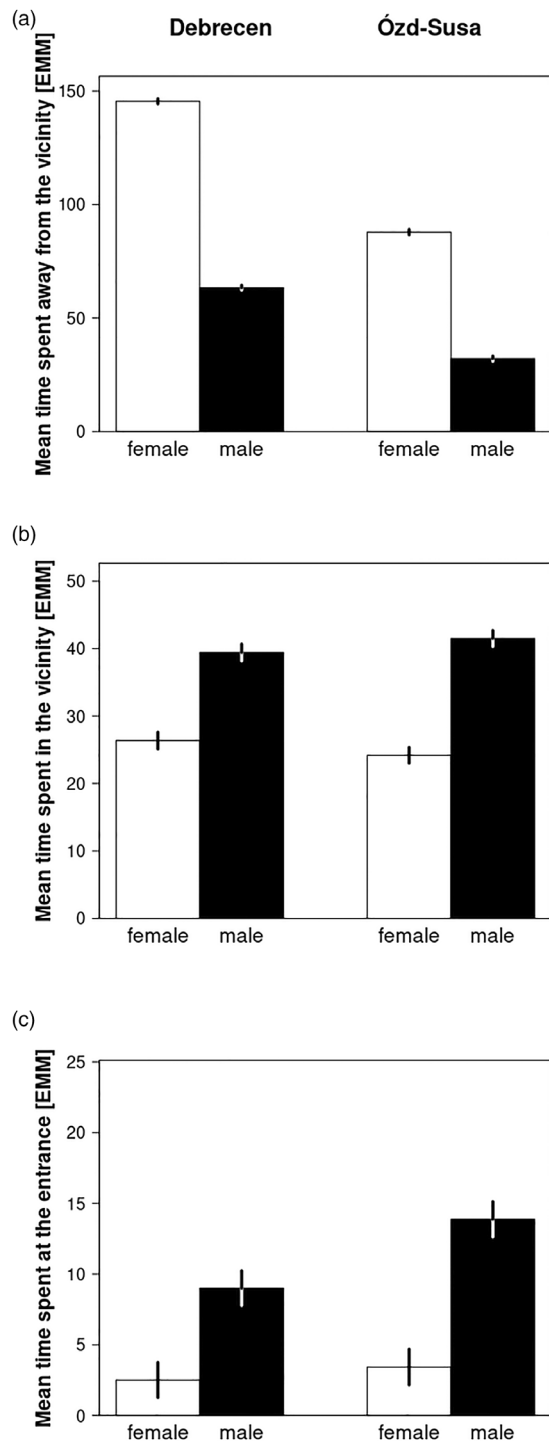
We found that ‘male status’ did not affect the number of offspring (negative binomial GLMM,  $\chi^2_1 = 0.117$ ,  $P = 0.733$ , Fig. 4). However, we found that ‘male status’ (i.e. unmarked new male or no male) affected the number of empty/unsuccessful brood chambers (negative binomial GLMM,  $\chi^2_1 = 8.670$ ,  $P = 0.003$ ). Accordingly, the number of unsuccessful brood chambers was significantly higher in nests without male residency (Fig. 4).

## Discussion

Our behavioural data confirm the findings of recent studies on parental care in *L. apterus* (Kiss et al., 2020; Kosztolányi



**Figure 1** Barplots illustrating *Lethrus apterus* females (white box) and males (black box) in rates (counts/h) of activities on the pre-treatment day of the Debrecen (2017) and Ózd-Susa population (2018). Investigated activities were: (a) rate of leaving the vicinity of the nest, (b) rate of being in the vicinity of the nest, (c) rate of emerging at the entrance of the nest, (d) rate of leaf collection and (e) rate of nest maintenance (i.e. pushing out soil from the nest). Estimated marginal means (EMMs) of activity rates are plotted. The error bars represent the SE of EMMs.



**Figure 2** Barplots illustrating differences between females (white box) and males (black box) in the duration (in seconds) of activities on pre-treatment day of the Debrecen (2017) and Ózd-Susa population (2018). Investigated activities were (a) mean time spent away from the vicinity of the nest, (b) mean time spent in the vicinity of the nest and (c) mean time spent at the entrance of the nest. Estimated marginal means (EMMs) of activity rates are plotted. The error bars represent the SE of EMMs transformed back from the original scale.

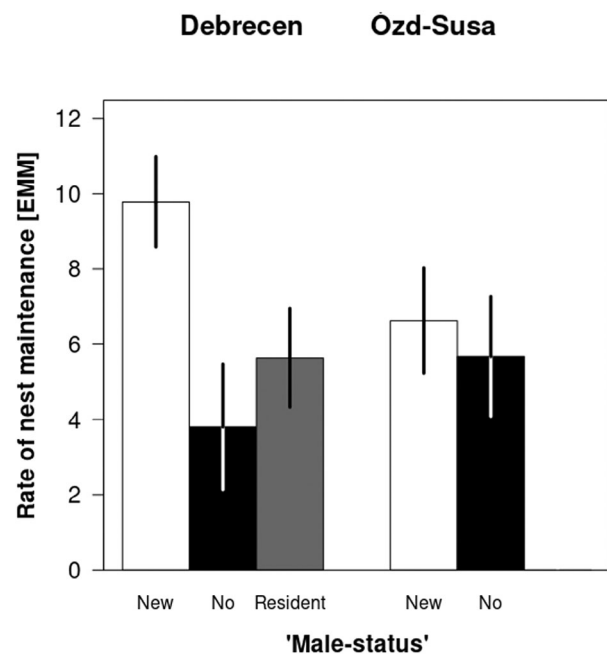
et al., 2015) suggesting that the primary task of males is nest-guarding, while females provide food for the larvae. Even at nests not manipulated, males deserted after a few days, and new males appeared soon and took over the position of the previous ones at the nests. Furthermore, we found no significant difference in care behaviour between females paired with a resident or a new mate or being without a mate. Females may not modify their care behaviour noticeably depending on the identity of their partner, indicating that there might be a strict division of tasks between the sexes (Barbasch et al., 2021; Henshaw et al., 2019). Our study also demonstrates that the behavioural repertoires of the sexes appear to be very similar in the two populations we studied.

We found that *L. apterus* males desert their nests rather quickly. In fact, in the Ózd-Susa population, no previously marked resident male was found at any of the nests by the third day. Males appeared to leave the nest either on their own accord or because of defeat in a fight (JK, RR and MER personal observation). So, our finding of quick desertion by males may indicate that rival males are more successful in taking over burrows (but see also Rosa et al., 2018) or that the resident male can gain higher fitness by deserting shortly after oviposition (Kokko & Jennions, 2012; Ward et al., 2009). The latter seems more probable because males might be better off searching and fighting for new, additional females with mature eggs than waiting for their partners to be sexually receptive again. Circumstantial evidence suggests that copulation precedes each oviposition. First, the oocytes of the females do not mature simultaneously suggesting that egg-laying occurs egg by egg (Nikolajev, 2003). Second, the egg of *L. apterus* is large compared to the size of the female, physically preventing the simultaneous development of eggs. Finally, females have poorly differentiated spermatheca, which is not expected to provide sperm storage and sperm release regulation during fertilization (López-Guerrero & Halfpeter, 2000). Consequently, the breeding season in *L. apterus* consists of several, more or less independent breeding cycles with subsequent events like copulation, nest expansion, oviposition, leaf-ball preparation and sealing the brood chamber with soil (Fig. 5; Nikolajev, 2003). Studies in deep burrowing geotrupid beetles (such as *Trypocoris vernalis*, *Typhaeus spp.*) seem to support the series of independent breeding cycles and the cooperation between the female and male in nest expansion (Brussaard, 1983; Hanski & Cambefort, 1991; Teichert, 1959a, 1959b). According to the study of Brussaard (1983), although the female does most of the burrowing in *Typhaeus typhoeus*, after copulation, the male also participates in the brood chamber construction. In *L. apterus*, each leaf ball, which is prepared as food for a larva, is produced from approximately 50 leaves or plant shoots (pers. obs., Frantsevich et al., 1977). *Lethrus apterus* beetles are active for an average of 5.5 h a day (Kiss et al., 2020), meaning that, based on our data, females need at least 2 days to collect a ball of leaves (Fig. 1d,  $4.89 \pm 0.64$  (mean  $\pm$  SE) leaves were collected per hour in Debrecen, and  $2.31 \pm 0.44$  (mean  $\pm$  SE) leaves were collected per hour in Ózd-Susa population). By adding the time required for the other phases of a nesting event, we expect that at least 3–4 days may elapse between two ovipositions (see also Brussaard, 1983; Hanski &

**Table 3** Results of the likelihood ratio tests for checking the significance of 'male status' as an explanatory variable (d.f. = 2, for all cases) in fitted models on rate (occurrence/h) and duration (in seconds) of activities related to parental care of females in two populations, in Debrecen (2017) and Ózd-Susa (2018)

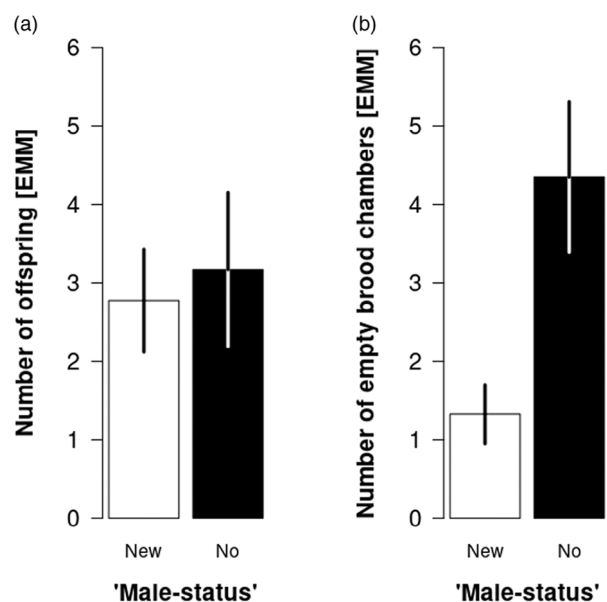
Activity trait	Debrecen		Ózd-Susa	
	$\chi^2$	<i>P</i> -value	$\chi^2$	<i>P</i> -value
Rate of leaving the vicinity of the nest	0.248	0.883	2.285	0.130
Rate of being in the vicinity of the nest	1.917	0.383	0.236	0.627
Rate of emerging at the entrance of the nest	1.159	0.560	1.177	0.278
Rate of leaf collection	0.111	0.946	1.514	0.219
Rate of nest maintenance	8.814	<b>0.012</b>	0.231	0.631
Mean duration of being away from the vicinity of the nest	1.302	0.522	0.211	0.646
Mean duration of staying in the vicinity of the nest	4.169	0.124	0.024	0.876
Mean duration of sitting at the entrance of the nest	4.024	0.134	0.308	0.579

Rate variables were investigated using negative binomial GLMMs, and duration variables using LMMs. Significant result is highlighted in bold.



**Figure 3** Barplot diagrams illustrating the rate (counts/h) of nest maintenance (i.e. pushing out soil from the nest) in females with different 'male status' (i.e. with new male (white box), no male (black box) or marked resident male (grey box)) from the post-treatment day of the Debrecen (2017) and Ózd-Susa population (2018). In the post-treatment part of the study on the second population, no female was found coupling with a resident male. Estimated marginal means (EMMs) of activity rates are plotted. The error bars represent the SE of EMMs.

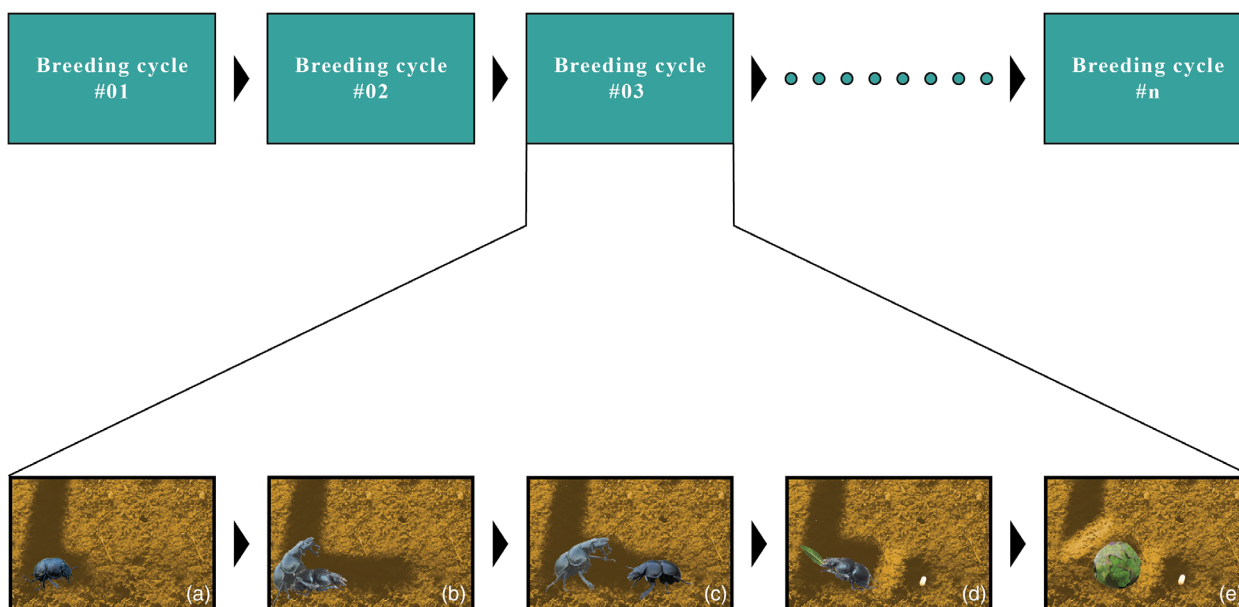
Cambefort, 1991; Verdú & Galante, 2004). Consequently, males might be better off by deserting soon after the females have laid the egg in favour to pursue other mating opportunities (Kokko & Jennions, 2008; Ward et al., 2009). The high number of intruders (75 fights were recorded on the video footages, in 80% of the nests in the Debrecen population; and



**Figure 4** Barplots illustrating (a) number of offspring and (b) number of empty brood chambers in nests of *Lethrus apterus*, with the presence of new unmarked males (white box) or no males (black box). Estimated marginal means (EMMs) are plotted; error bars represent the SE of EMMs.

24 fights, in 45.5% of the nests in the Ózd-Susa population) also support our notion that many males are looking for further mating opportunities with other females (Alonzo & Klug, 2012; Kokko & Jennions, 2008; Webb et al., 1999). Nevertheless, the finding that males left the nest after a few days or less implies that females most likely mate with multiple males during a reproductive season, a prediction awaiting a future genetic parentage test.

We found that *L. apterus* males spend much time at the entrance of the nest (most likely to keep intruders and predators away), while the females provision the brood cells with plant materials collected and carried from the surrounding



**Figure 5** Breeding cycle of *Lethrus apterus* beetles based on observations and the family characteristics. The five panels illustrate the supposed main subsequent events of a cycle. (a) The female excavates the brood chamber branching off the main tunnel. (b) The male joins in the tunnel, the pair copulate. (c) While the female lays the egg, the male expands and widens the brood chamber. (d) The female seals the egg cavity with soil and collects plant material to form a leaf ball for the larva. (e) The brood chamber is sealed with soil after the leaf ball is prepared by the female.

vegetation. Although females seemed to be predominant in nest construction, males also took their share. Removal of soil from the nest by males may indicate involvement in brood chamber excavation, which would also confirm the idea of cooperation between the parents in nest expansion. In onthophagine beetles, sexes cooperate in dung collection and brood ball construction for the larvae (Hunt & Simmons, 2002a; Simmons & Ridsdill-Smith, 2011), as males help in collecting and transporting dung from the dungpad to the nest, while females spend more time to process dung and form balls for the larvae inside the nest (Hunt & Simmons, 2002a). The notion that in *Ontophagus* species, both sexes participate in providing food for the larvae, but not in *L. apterus*, can be explained by the limited and ephemeral availability of dung as a source compared to more plentiful plant materials (Hanski & Cambefort, 1991; Simmons & Ridsdill-Smith, 2011; Verdú & Galante, 2004). Additionally, in species with intense male–male competition, as in the case of *L. apterus* beetles (Nikolajev, 2003; Rosa et al., 2018), vigorous nest defence behaviour can result in a correlated decline in the amount of other care activities that males could provide (Hunt & Simmons, 2002b; Schrader et al., 2020).

Although the same trend was observed in the activity pattern of the sexes in both populations, the individuals from the Ózd-Susa population seemed to be less active. While the sample sizes in the Ózd-Susa population were lower, these subtle differences might be explained also by behavioural variation among years or populations due to different weather and other ecological conditions (Ratz et al., 2016; Wong et al., 2013). For example, insects may avoid adverse temperatures by behavioural

mechanisms, for example, searching for and staying in optimal microclimate and reducing activity during adverse periods (Gullan & Cranston, 2014; Smolka et al., 2012). Furthermore, temperature and humidity can affect the breeding activities of the individuals as well as the amount of energy and time invested in nest construction (Holley & Andrew, 2018; Macagno et al., 2016; Mamantov & Sheldon, 2021; Sowig, 1996).

We found that females in *L. apterus* did not differ in the level of their parental investment when their partners deserted or were experimentally removed. However, the majority of the females did not remain alone for long after their partners were gone. In several burying beetle species, partners flexibly share the tasks of caring for offspring (Benowitz et al., 2016; Rauter & Moore, 2004; Smiseth et al., 2005). Thus, parents can cooperate in performing the same tasks and compensate for the partner's effort in case of mate removal (Creighton et al., 2015; Fetherston et al., 1994; Smiseth et al., 2005; Suzuki & Nagano, 2009). On the contrary, in *L. apterus*, the roles of the sexes seem to be more fixed, with females providing most of the care for the offspring and males guarding the entrance of the tunnels. Moreover, it is likely that males desert before the leaf balls are prepared for their offspring by their mates, in which case, females should provide all provisioning as males do not contribute significantly to the well-being of the offspring. Consequently, females may not be expected to alter their parental behaviour in case of male desertion. Nonetheless, further studies, such as female removal experiments, are required to ascertain whether the males do care for offspring in the absence of the females or not.

Based on the observed frequent mate changes in *L. apterus*, each laid egg might be fertilized by a different male, which is supported by studies on the female reproductive system of *Lethrus* species (López-Guerrero & Halfiter, 2000; Nikolaev, 2003), and males may desert the female soon after successful fertilization (i.e. males may perform 'pre-oviposition' guarding). Our result, that females paired with new partners removed soil from the nest at a significantly higher rate, might indicate their preparation for oviposition preceded by building a new brood chamber. Conversely, the finding that females paired with resident males (or no males) invested less in nest maintenance might indicate that they had already excavated the brood chamber and prepared for or completed oviposition (or have not mated yet, in the case of lone females). On the other hand, in the Ózd-Susa population, we found no significant difference between females with new or no partners in nest maintenance after the treatment. However, trends were similar to those of the Debrecen population and the lack of significant effect might be traced back to the lower sample size in Ózd-Susa population. It is also possible that since we observed the nests 1 day after the treatment in the Ózd-Susa population, the females with new partners might have been close to the end of brood chamber excavation by then. Nevertheless, the details of the underground behaviour of *L. apterus* beetles are still largely unknown.

Our results showed that the absence or presence of the male partner had no significant effect on the offspring number but did affect the number of unsuccessful chambers. Specifically, nests of the Ózd-Susa population, where no males were found on the post-treatment day, had a significantly higher number of unsuccessful chambers. The lack of mate guarding may have contributed to the reduced survival of offspring (Gilbert et al., 2010; Requena et al., 2009). By guarding, the males can keep not only rivals but also parasites and predators away from the nest (Suzuki, 2013). It has been reported in several insect species with parental care, that conspecific intruders, but in some cases the parent, tend to kill resident larvae to gain reproductive benefits (Huerta et al., 2003; King & Fashing, 2007; Koulianos & Schwarz, 2000; Trumbo, 2006). Nevertheless, we have no information about ovicide or larvicide behaviour in *L. apterus* beetles. Overall, despite this, we have no information about the females' parental efforts nor the actions between the end of the experiment and the nest excavation. Thus, we do not consider these results to be a good proxy for explaining differences in reproductive success in the case of male presence or absence.

In conclusion, we show for the first time that *L. apterus* beetles appear to have a particularly dynamic mating system with typically short-term mate fidelity, while the roles of the sexes are rather stable. Females seemed to be solely responsible for provisioning, and they did not change their behaviour dramatically after the experimental removal of their partner. Males spent most of their time guarding the nest and fighting with rival males, but it remains unclear whether this behaviour is beneficial solely for the male or also for the offspring. Overall, our results suggest that contrary to previous characterization as a monogamous system (see Hamilton, 1979), *L. apterus* may have a sequential polygamy mating system. Nevertheless, it will be of high interest to examine the underground activities

of the sexes to get a clearer picture of care behaviour in *L. apterus* beetles.

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## Author contributions

JK, MER, RR, AK, and ZB conceived the ideas and designed research; JK, MER, and RR performed research; JK and ZB analyzed data; and JK wrote the manuscript with input from all authors.

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