

# **Variation in the onset of parental behaviours across four species of *Peromyscus* mice with different mating systems**

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## ABSTRACT

Parental care is defined as any parental investment that results in an increase in offspring fitness and a reduction in parental reproductive potential. Although observed across the animal kingdom from insects to mammals, parental behaviours show great inter- and intra-specific variation in nature, intensity and timing. In biparental species, the mother and the father usually form a long-term association and rear their young together. Although both sexes can display parental behaviours, it may not be beneficial for them to be parental all their lives, and the onset of parental behaviours could be timed precisely.

The wild mouse genus *Peromyscus* is an ideal model to study the evolution of parental care, as monogamy and biparental care have evolved independently at least twice in the genus. In this study, I compared the parental behaviours of males and females of four *Peromyscus* species at four reproductive stages: before mating, after mating, at late pregnancy and after giving birth. *Peromyscus maniculatus* and *Peromyscus leucopus* are promiscuous and uniparental, which was reflected by parental behaviours being mostly displayed by mothers. *Peromyscus polionotus* and *Peromyscus californicus* are monogamous and biparental, and both sexes showed parental behaviours, but with different timings and intensities. The former species seemed to be parental at all reproductive stages, with females being more parental than males. In contrast, the latter species was mostly parental after birth of the young, and both sexes showed equivalent amounts of parental behaviours. Infanticidal behaviours were mostly displayed by *P. californicus* before the birth of their young. These differences in the onset of parental behaviours are best explained by species differences in territoriality and dispersal, and highlight the independent evolution of biparental care and monogamy in this genus.

Keywords: parental behaviours, onset, *Peromyscus*, mating systems

## INTRODUCTION

Animal parental care is defined as any parental investment in resources that results in an increase in offspring fitness and that incurs a cost to parental future reproduction (Kleiman and Malcolm 1981; Clutton-Brock 1991). It includes many forms of indirect (e.g. territory defence, nest building) and direct care (e.g. feeding, grooming, teaching) and is widespread across the animal kingdom, from insects to humans (Clutton-Brock 1991; Dulac et al. 2014).

Although commonly observed in all vertebrate classes, parental care and behaviours show great variation in their nature, intensity and allocation between parents. The balance of care between mother and father depends, among other factors, on parentage certainty and thus, on the fertilization mode (Clutton-Brock 1991; Elwood 1994). In internal fertilizers like mammals, multiple inseminations are possible, and thus fathers have a relatively low paternity certainty, while mothers usually only have their own offspring to care for from birth to weaning (Elwood 1994). Furthermore, a long association between the mother and her offspring is obligatory in mammals due to lactation, which imposes a physiological constraint on females to care for their offspring for an extended period of time after birth (Kleiman and Malcolm 1981). On the other hand, males are typically free to desert during gestation and lactation. It is thus more advantageous for them to seek new mating opportunities than to wait and care for their mate's litter (Clutton-Brock 1991; Gubernick and Teferit 2000). As a result, it is still widely accepted that paternal care is relatively rare among mammals (Kleiman 1977; Kleiman and Malcolm 1981; Stockley and Hobson 2016).

However, paternal care has evolved numerous times independently in 10% of mammalian genera and is often associated with a monogamous mating system, where mother and father pair up for life and rear their offspring together (Kleiman 1977; Kleiman and Malcolm 1981; Stockley and Hobson 2016). The observed prevalence of monogamy and biparental care in mammals has been increasing since research switched its focus to smaller, nocturnal species (Foltz 1981; Stockley and Hobson 2016). In rodents, males sometimes participate in all forms of care but nursing, and their care can significantly increase the survival of the offspring (e.g. Gubernick and Teferit 2000). The similarities and

differences in parental behaviours between sexes has been reviewed, with a focus on laboratory rodents (Lonstein and De Vries 2000).

Although both males and females can display parental behaviours, they would not necessarily benefit from being "parental" all their lives (Lonstein and De Vries 2000). In theory, the onset of parental behaviours within an animal's life should be timed as precisely as physiologically possible in order to (1) avoid the cost of caring for unrelated young but also to (2) avoid the cost of not caring/being aggressive towards one's own offspring or closely related young (Elwood 1994). In laboratory rodents (e.g. *Mus*, *Rattus*), it is well documented that virgin males tend to be non-parental or even infanticidal towards pups until they first mate (Perrigo et al. 1990; Brown 1993; Elwood 1994; Dulac et al. 2014). Wild rodent females show a similar trend and only behave maternally with their own pups, or with pups born in their home range during the lactation period (McCarthy and vom Saal 1985; Soroaker and Terkel 1988; Wolff and Cicirello 1991). Although this pattern has been repeatedly observed, the timing of the onset of parental behaviours (and the inhibition of infanticide) is subject to substantial inter- and intra-specific variation, and can be influenced by several factors such as an individual's reproductive stage, its social environment and prior exposure to juveniles (McCarthy and vom Saal 1985; Gubernick et al. 1994; Dulac et al. 2014).

To fully understand the evolution of proximate mechanisms underlying the onset of parental behaviours, it is important to examine several species that differ in their mating and parental systems using a common methodology (Gubernick et al. 1994). The present study aims at exploring the timing of the onset of parental behaviours in four mouse species of the genus *Peromyscus*.

The murid mouse genus *Peromyscus* is widespread in North America and has become an important mammalian model organism for the evolution of social behaviours, because it displays a variety of social and mating systems and is easy to study both in the wild and in captivity (Bedford and Hoekstra 2015). Although the ancestral mating system is considered to be promiscuity associated with maternal care only, monogamy and biparental care have evolved at least twice independently within the genus (Fig. 1), which makes it an ideal model to investigate the proximate mechanisms behind the evolution of sex-specific parental behaviours. From previous work, it is known that *Peromyscus maniculatus bairdii* (hereafter *P. maniculatus*; Fig. 1) and *Peromyscus leucopus* (*P. leucopus*; Fig. 1) have a

polygynous/promiscuous mating system where only the mother maintains a close association with and takes care of the offspring for several weeks after birth (Dewsbury 1981; Xia and Millar 1988; Wolff 1989; Wolff and Cicirello 1991; Jašarević et al. 2012). In contrast, *Peromyscus polionotus subgriseus* (*P. polionotus*; Fig. 1) is a well-known monogamous subspecies with stable mating pairs and biparental cooperation in offspring rearing (Foltz 1981; Wolff 1989; Jašarević et al. 2012; Bendesky et al. 2017). Finally, *Peromyscus californicus insignis* (*P. californicus*; Fig. 1E) is strongly territorial, and is considered to be exclusively monogamous, where lifelong pairs form and show biparental care (Dewsbury 1981; Ribble 1991; Gubernick and Teferit 2000; Jašarević et al. 2012). Males of this species display all parental behaviours (except nursing) to a comparable extent to females, and seem to play a more important role than *P. polionotus* males, as their absence significantly impacts on offspring survival (Margulis 1998; Gubernick and Teferit 2000; Margulis et al. 2004).

Using the above-mentioned four species in controlled laboratory conditions, I measured the parental behaviours and attraction to pups of individual mating pairs over four reproductive stages: as virgins, after copulation, at late pregnancy and after giving birth. I stated the following general hypotheses:

- (1) *P. maniculatus* and *P. leucopus* are promiscuous, so males have a low paternity certainty in the wild. Thus, only females display parental behaviours, and only after giving birth. If any paternal care is displayed, males spend less time caring than females in both species. Mothers are more attracted to pups than other mice.
- (2) *P. polionotus* and *P. californicus* are monogamous, so males have a high paternity certainty in the wild. Thus, both sexes show parental behaviours after their pups are born. Parents are more attracted to pups than mice at earlier reproductive stages.

## MATERIALS AND METHODS

### *Animal husbandry*

I obtained virgin animals from the established colonies of *Peromyscus maniculatus bairdii* (strain BW), *Peromyscus leucopus leucopus* (strain LL), *Peromyscus polionotus subgriseus* (strain PO), and *Peromyscus californicus insignis* (strain IS). These colonies were originally established from animals obtained from the *Peromyscus* Genetic Stock Center at the University of South Carolina, USA. The average $\pm$ SD age of an animal when tested as a virgin was of 180 $\pm$ 64 days, the oldest animal being 401 days old and the youngest one 74 days old. They were housed in specific pathogen-free conditions with 16h light: 8h dark at 22°C. Smaller strains (BW, LL, PO) were housed in individually-ventilated Allentown cages (28.5cm long x 19cm wide x 16cm high; Allentown, Allentown, NJ, USA) with ~1cm Anderson's Bed-o-cob bedding (The Andersons, Inc., Maumee, OH, USA), while the larger strain (IS) was housed in larger Allentown cages (43cm long x 22cm wide x 27cm high) with ~3cm of bedding. Virgins were housed in social groups of two to five individuals of the same strain and sex, were fed *ad libitum* with LabDiet Prolab Isopro RMH 3000 5P75 (LabDiet, St. Louis, MO, USA), and had unlimited access to water. Breeding pairs and their litters were fed irradiated PicoLab Mouse Diet 20 5058 (LabDiet, St Louis, MO, USA) *ad libitum* and had free access to water as well. I equipped all cages with two/four (5g/10g total, for smaller strains/larger strain, respectively) squares of compacted cotton (Nestlet, Ancare, Bellmore, NY, USA), ~10g/30g (smaller strains/larger strain, respectively) of paper fibre nesting material (Enviro-Dri, LBS Serving Biotechnology, UK) and a polycarbonate translucent red hut/tunnel (smaller strains/larger strain, respectively). This project falls within the scope of a protocol approved by the Harvard FAS IACUC.

### *Parental behaviour test*

The assay described here is based on the methodology by Bendesky and co-workers (Bendesky et al. 2017).

I conducted this test during the light phase of the daily cycle, and not within 30 minutes of the light switch. Preliminary data did not show much difference between tests performed during the dark/light

phases, so I decided to follow Bendesky et al. (2017) (see Supplementary material). I transferred the animals to a testing room at least 5 minutes prior to the start of the test while leaving them in their home cage. The nest, red hut, food hopper and all animals but the test animal were transferred to a new cage and left undisturbed ~3m from the test animal's cage. When I tested breeding pairs, I always tested the female first in her home cage, while the male was later tested in the cage in which he had been transferred, after at least 30 minutes of habituation. The female was tested first because preliminary tests indicated that females may be more subject to stress (stereotyped backflipping) than males (data not shown). I decided not to test males in their home cage in order to avoid the extra stress of a second cage transfer.

To begin each test, I gave the test individual ~0.625g/2.5g (smaller strains/larger strain, respectively) of compacted cotton nesting material (Ancare, Bellmore, NY) (time 0). At 30 minutes, I scored the quality of the nest according to the scale described below, and then placed an unfamiliar conspecific pup inside the cage, ~20cm away from both the nest and the test individual. The pups were 2-8 days of age (when possible restricted to 4-5, depending on the availability in the colony). Then, I scored the latency to approach the pup, to handle the pup with the front paws, to lick the pup, to huddle over the pup (i.e. when the test individual covers at least 50% of the pup's body with its own body) and to retrieve the pup (i.e. when the test individual picks up the pup with its mouth and displaces it). If the test individual did not handle, lick, or huddle over the pup, it was attributed the maximal value (1200 seconds) for the latency to show these behaviours. I also scored the total time spent licking and huddling over the pup until minute 50, point at which I scored the nest quality again. At minute 50 (20 minutes after adding the pup), I removed the pup for 10 seconds and added it back to the cage for 2 minutes (with the same placement guidelines as above) to measure the latency to retrieve again. I repeated this procedure twice more before the test ended.

I scored each test ~100-150cm away from the cage by observing the cage directly while video-recording the test from the opposite side for later verifications when the displayed behaviour was not clear. In the event of an infanticidal attack (the test individual jumps onto the pup and bites it), I immediately interrupted the test by hitting the lid of the cage (which disrupted the attack) and removed the pup. Attacks were confirmed with the presence of bite marks or blood on the pup. This procedure

allowed to save all but two pups who were instantly killed by the test individual. I recorded the outcome of a test as being aggressive or not. When possible, the same pup was not used for two consecutive tests. When the availability of pups did not leave me another option, I left the pup for 10 minutes in an incubator at 37°C between two tests. At the end of the testing bout, I returned the pup to its parents and ensured that it was taken care of (licked and/or retrieved by a parent).

#### *Nest quality score*

I used the same scoring system as Bendesky and colleagues (Bendesky et al. 2017) to quantify nest-building behaviour: 0 = nesting material is untouched; 1 = all of the nesting material is shredded and scattered; 2 = nesting material is shredded and gathered in a platform; 3 = nesting material is shredded, gathered in a nest and forms walls that are as high as the test animal. Nests that were between two discrete categories were attributed an intermediate score.

#### *Test of attraction/aversion to a pup*

I conducted this test during the dark phase of the daily cycle (for it not to overlap with the parental behaviour test), and not within 30 minutes of the light switch. I designed a 47cm x 47cm x 35cm square arena using laser-cut cast acrylic (Figure 2A). The arena had a square lid which edges were lined with IR LED strips to illuminate the arena's floor. I subdivided the arena into five compartments: one central compartment and four peripheral compartments. The central compartment had its own lid and four sliding doors that could be opened remotely and simultaneously. An upturned pencil holder was placed in the center of each of the four peripheral compartments (Figure 2B). The pencil holder's wall consisted of evenly spaced metallic bars, allowing air and sound to travel through freely, but preventing the test mouse to reach the holder's content. Each pencil holder hid a plastic weighing dish with a specific content. One contained ~8g of peanut butter (Teddies All Natural Peanut Butter, The Leavitt Corporation, Everett, MA, USA), another contained an unfamiliar conspecific pup of 2-7 days of age, and the last two were empty and were used as controls. I randomized the attribution of each holder content to one peripheral compartment of the arena for each test.



To start the test, I transferred the test individual from the housing room in a cardboard box to the test room, which was only lit by a red light. I immediately released the test mouse into the closed central compartment of the arena and left it there for 2 minutes for it to calm down. After this acclimation period was over, I launched a video-recording script which would start recording 1 minute later, for 10 minutes<sup>1</sup> in total. I immediately removed the central compartment's lid, opened the sliding doors, put the IR LED-equipped lid onto the arena, turned off the red light and left the room. The animal was left to move freely in the arena during the video-recording period and its movements were recorded from above, using a Raspberry Pi 3 Model B, a Pi Camera NoIR (Raspberry Pi Foundation, UK) and a python script provided in the Supplementary material ("Attraction-aversion\_test.py"). After the test was done, I came back to the test room and returned the test animal to the housing room the same way I brought it in. Before another test was run, I cleared the arena of droppings, and wiped all horizontal surfaces with 70% ethanol to eliminate urine traces. A pup was never used twice in a row and was thus allowed at least 15 minutes in an incubator at 37°C before it was used for another test.

#### *Experimental timeframe and pairing*

All experimental animals were subject to both behavioural tests at four reproductive stages: as sexually naive animals ("virgin"), after copulation had happened or was expected to have happened ("mated"), when the female was expected to be in late pregnancy ("expecting") and after birth of the pups ("parent"). If a pair's first litter did not survive more than three days, I tested the parents after the second litter was born.

The parental behaviour test was done the day following the corresponding attraction/aversion test.

Between 1 December 2016 and 17 February 2017, I paired 12 virgin females of each strain to 12 virgin males of the same strain anytime after they had been tested for the first time, and left them paired until the end of the experimental period (21 March 2017). I monitored mating for three nights after pairing, using the procedure described below. If I witnessed mating (see *Mating monitoring* below), the second series of tests was performed on days 1-2 post-mating. If I did not see any evidence

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<sup>1</sup> Initially that time was 20, but was later reduced as a longer time did not seem to bring any extra information (see Supplementary material).

of mating, tests were performed after 5 nights (smaller strains) or 6-7 nights (larger strain), as this represents the approximate duration of an estrus cycle for these species. The third test bout was done on days ~20-21 after mating (after the 5th night if mating was not observed) (smaller strains) or on days ~27-28 after mating (after the 6th night if mating was not observed) (larger strain). The final tests were run 2-6 days after the first surviving litter was born. I excluded all pairs that did not give birth during the experimental period from the statistical analysis, but used them to describe infanticidal behaviours.

As it was not clear at the beginning of the project whether the experimental period was long enough for enough new pairs to give birth, the existing breeding pairs of the colonies of LL (7 pairs), PO (7 pairs) and IS (8 pairs) were tested 2-6 days after one of their litters was born ("parent only"). BW is known to breed reliably in the lab and was therefore not tested as parents only. This back up plan would have allowed to compare virgin individuals to parent individuals, without repeated measurements.

### *Mating monitoring*

As *Peromyscus* are more recently wild-derived than most laboratory rodents, they have been subject to a lower amount of selection pressure to become tame. Handling them repeatedly may thus cause substantial stress, hinder a successful pregnancy and alter their parental behaviours (Kyle Turner, personal communication). Thus, I decided to avoid frequent manipulations/palpations of paired females to confirm mating/late pregnancy and monitored reproductive stages indirectly.

I monitored mating using a home-made camera setup using the Raspberry Pi Zero and a Raspberry Pi NoIR camera (Raspberry Pi Foundation, UK) mounted on the cages and powered by a battery pack. For further details on how I constructed the setup, see Supplementary material. The Raspberry Pi was programmed to record 8.25 hours of video from 5 minutes before the light turns off to 10 minutes after the light turns on. As all study species are nocturnal (Bedford and Hoekstra 2015), it was assumed they would mate at night. I extracted the videos from the Raspberry Pi and watched them in accelerated mode (4-8x) in the next days, with the help of Andrés Bendesky. The sought-after components of mating behaviour were: chasing (the male runs after the female), mounting (the male

climbs onto the female's back), thrusting (the male's hips move back and forth) and post-coital licking (the male and sometimes the female lick their genitals after the male ejaculated) (see an exemplary video in Supplementary material ("Pmaniculatus\_normal-mating.h264")). I considered mating to have occurred either if all the components were observed at least once, or if mounting and thrusting were observed repeatedly overnight.

### *Data analysis*

#### Attraction/aversion video analysis and test comparison

I tracked the movements of test mice using the software Ethovision XT 11.5 (Noldus et al. 2001). The arena settings (background image and zones) were adjusted for each video as cleaning the arena made it slightly move between each test. The detection settings for most videos were the following:

- Method: Dynamic Subtraction; Model-based (XT 5); Dark: 44-184; Frameweight: 29
- Smoothing: Video Pixel Smoothing: None; Track Noise Reduction: on
- Subject Contour: Subject Size Maximum: 125000

The detection settings were tested on each video to make sure the mouse was correctly tracked. In case tracking did not work well, I adjusted the detection settings for that specific video.

From the videos, I measured the time spent in each zone and calculated the following variables, used later on in the statistical analysis:

(1) Proportion of time spent in the pup zone:

$$\frac{t_{pup}}{t_{pup} + t_{peanut\ butter} + t_{controls}}$$

where  $t_x$  is the time spent in the zone containing x.

(2) Preference index between pup and peanut butter zones:

$$\frac{t_{pup} - t_{peanut\ butter}}{t_{pup} + t_{peanut\ butter}}$$

Where a preference index  $\rightarrow 1$  indicates a stronger preference for the pup zone, while a preference index  $\rightarrow -1$  indicates a stronger preference for the peanut butter zone.

To compare the results of the test of attraction/aversion to a pup and the parental behaviour test, I made up a proxy for attraction/aversion from the parental behaviour data. The "latency of physical contact after first approach" was calculated as the difference between the shortest latency of physical contact (between latency to handle, lick, huddle over, retrieve or attack the pup) and the latency to approach the pup. If a test mouse never approached the pup, it was attributed the maximal value for the latency of physical contact after first approach (1200 seconds).

### Statistical analysis

All statistical analyses were performed in the R language and environment (RCoreTeam 2016). All behaviours were analyzed in a similar fashion, using a general linear mixed-effects model approach, with the *lmer* function from the lme4 package (Bates et al. 2015). In the lme4 syntax, the models were of the following general form:

$$behaviour \sim species + sex + reproductive\ stage + 2way\ interactions + (1|ID)$$

where all explanatory variables are factorial, individual ID is a random-effect factor, and the response variable can be subject to transformation if the model's residuals do not follow a normal distribution (log(x+1) for time spent huddling, time spent licking and latency of physical contact, log(x+0.1) for nest quality score,  $\sqrt{x}$  for proportion of time spent in pup zone). The normality of the model's residual distribution was assessed graphically and if necessary with a Shapiro-Wilk test ( $p > 0.05$ ). A three-way interaction term was not included in the models as its interpretation is not trivial.

The significance of fixed effects was determined using a Likelihood Ratio test with the *anova* function (RCoreTeam 2016), comparing the full model with a simplified model where the fixed effect of interest was taken out. The output of this function includes a  $\chi^2$ -based p-value. To reinforce the meaning of this p-value, I used the Akaike Information Criterion (AIC) to compare models within pairs:

$$\Delta AIC = AIC_{\text{model without fixed effect}} - AIC_{\text{model with fixed effects}}$$

When this difference  $> 0$ , the model with the fixed effects has a better goodness of fit than the null model.

When a fixed effect was not significant, it was removed from the model before testing the next fixed effect in order to gradually select the best model. The significance of fixed effects was tested in the following order: *Species : Sex*, *Species : Reproductive Stage*, *Sex : Reproductive Stage*, *Species*, *Sex*, *State*. When one or more interaction terms were significant, I decided not to assess the significance of main effects.

Significant fixed effects were further explored independently with post-hoc comparisons using the *glht* function from the multcomp package (Hothorn et al. 2008). The *p*-values of the multiple comparisons were adjusted with a Bonferroni-Holm correction for multiple testing (parameter *test = adjusted("holm")*).

#### Habituation to the test of attraction/aversion to a pup

As a control for the effect of habituation to the test of attraction/aversion to a pup, experimental parents (mice that were tested at four reproductive stages) and parents only (mice that were only tested as parents) were compared in their proportion of time spent in the pup zone and their preference index. This was done with linear models after checking for the normality of the model's residual distribution (see above) and for the homogeneity of group variances (Bartlett test,  $p > 0.05$ ). The selection of the best model was done following the same procedure as described above, except that the difference in Residual Sum of Squares (RSS) was used as an indicator of model improvement instead of  $\Delta AIC$ .

## RESULTS

Within the experimental period, 8 pairs of *P. maniculatus*, 9 pairs of *P. leucopus*, 8 pairs of *P. polionotus*, and 7 pairs of *P. californicus* gave birth to a litter that lived for more than three days, and were thus tested at all reproductive stages. Of these 32 pairs, 22 were tested as "expecting" 10 days or less before they actually gave birth, 5 may have been in late pregnancy of an unsuccessful litter, and 5 pairs were not in late pregnancy (calculated based on a 24-day/30-day gestation time for smaller strains/larger strain, respectively). This limits the precision of my interpretation of reproductive stages.

## Parental behaviours

### Time spent huddling

The amount of time that a test mouse spent huddling over an unfamiliar pup was significantly influenced by (1) a *Species : Reproductive Stage* interaction, and (2) a *Sex : Reproductive Stage* interaction (Table 1). Indeed, the goodness of fit of the mixed-effects model was significantly worsened when removing these two terms. Model estimates, their standard error and  $z$  values are given in Table 2.

(1) As visually assessed and given by post-hoc comparisons (Figure 3, Table 3), *P. maniculatus* and *P. leucopus* mothers, as well as *P. polionotus* and *P. californicus* parents spent a relatively long portion of the test huddling over the pup. Furthermore, virgin, mated and expecting *P. maniculatus* spent significantly less time huddling than most other *Species : Reproductive Stage* combinations. Finally, *P. polionotus* spent a relatively long time huddling at all reproductive stages, which was not the case in *P. californicus*.

(2) Overall, mothers spent significantly more time huddling over the pup than all other *Sex : Reproductive Stage* combinations (Figure 3, Table 4). Also, fathers spent more time huddling than virgin males, mostly in biparental species.

### Time spent licking

The amount of time that a test mouse spent licking an unfamiliar pup was significantly influenced by a *Species : Reproductive Stage* interaction (Table 1). Model estimates, their standard error and  $z$  values are given in Table 2.

At all reproductive stages, *P. maniculatus* showed very little licking as compared to other *Species : Reproductive Stage* combinations (Figure 4, Table 5). Similarly, mated and expecting *P. leucopus* as well as virgin *P. californicus* spent a relatively low amount of time licking the pup. In contrast, *P. polionotus* at all stages and *P. californicus* parents spent a high amount of time licking the pup. Interestingly, *P. polionotus* females spent more time licking the pup before than after giving birth,

which almost led the *Species : Sex* interaction term to significantly affect the model's goodness of fit (Table 1).

#### Proportion retrieved

The proportion of times the test mouse retrieved the unfamiliar pup (out of four times) was significantly influenced by (1) a *Species : Reproductive Stage* interaction and (2) a *Sex : Reproductive Stage* interaction (Table 1). Model estimates, their standard error and *z* values are given in Table 2.

(1) *P. maniculatus* at all stages retrieved a remarkably low number of times when compared to other *Species : Reproductive Stage* combinations (Figure 5, Table 6). In contrast, *P. leucopus* showed an intermediate proportion until they became parents, where both mothers and fathers retrieved a relatively high amount of times. In general, parents retrieved more often than other stages, but the increase in the proportion retrieved across reproductive stages was smoother in *P. leucopus*, *P. polionotus* and *P. californicus* than in *P. maniculatus*, where only mothers show a high proportion of retrieved pups.

(2) Similarly to the time spent huddling, mothers significantly differed from all other *Sex : Reproductive Stage* combinations and retrieved the unfamiliar pup almost every time (Figure 5, Table 7). Fathers also retrieved a significantly higher number of times than virgin males, as visible in all species but *P. maniculatus*.

#### Nest quality score

The quality score of the nest built by the test mouse after 20 minutes of exposure to an unfamiliar pup was significantly influenced by a *Sex : Reproductive Stage* interaction (Table 1). Model estimates, their standard error and *z* values are given in Table 2.

Across all reproductive stages, males showed very little nest building, except *P. californicus* males who increased their nest quality as they became fathers (Figure 6, Table 8). In contrast, expecting females and mothers built significantly better nests than most other *Sex : Reproductive Stage* combinations, with most *P. leucopus*, *P. polionotus* and *P. californicus* mothers building nests of score 2 or higher.

#### Latency of physical contact after first approach

The time that a test mouse took to physically interact with the pup (handle, lick, huddle, retrieve or attack) after first approaching it was significantly influenced by (1) a *Species : Reproductive Stage* interaction and (2) a *Sex : Reproductive Stage* interaction (Table 9). Model estimates, their standard error and  $z$  values are given in Table 10.

(1) Virgin, mated and expecting *P. maniculatus* stand out from all the stages of other species (Figure 7, Table 11), as many individuals either never approached the pup or never interacted with it after the first approach. Interestingly, some *P. californicus* showed a gradual decrease in latency of physical contact across reproductive stages, which was not observed in other species.

(2) Females physically interacted with the pup more quickly as mothers than before giving birth, and also more quickly than mated, expecting and parent males (Figure 7, Table 12) who tended to stay away from or ignore the unfamiliar pup.

#### Proportion of time spent in the pup zone

The proportion of time (out of 10 minutes) spent in the zone where the holder hid an unfamiliar pup was significantly influenced by the *Reproductive Stage* (Table 9). Model estimates, their standard error and  $z$  values are given in Table 10.

Virgin individuals spent a significantly higher proportion of their time (mean $\pm$ SD = 0.32 $\pm$ 0.12) close to the pup than expecting mice (0.25 $\pm$ 0.08) (Figure 8, Table 13).

#### Preference index

Similarly, the preference index between the pup zone and the peanut butter zone was significantly influenced by the *Reproductive Stage* (Table 9). Model estimates, their standard error and  $z$  values are given in Table 10.



Virgin individuals had a significantly higher preference index ( $0.21 \pm 0.31$ ) than all other reproductive stages (mated:  $0.04 \pm 0.29$ , expecting:  $-0.001 \pm 0.23$ , parents:  $0.08 \pm 0.29$ ), indicating a tendency to have a higher preference for the pup zone over the peanut butter zone (Figure 9, Table 13).

#### *Habituation to the test of attraction/aversion to a pup*

The proportion of time spent in each zone category (pup, peanut butter or empty) by *P. leucopus*, *P. polionotus* and *P. californicus* as virgins and parents, as well as individuals tested only as parents is represented on Figure 10.

#### Proportion of time spent in the pup zone

When parents and parents only are compared in a linear model (data untransformed), *Reproductive Stage* significantly influenced the proportion of time spent in the pup zone, and reduced the residual variation of the data (Table 14).

Individuals tested only as parents spent a significantly higher proportion of their time close to the pup ( $0.34 \pm 0.10$ ) than parents tested at all reproductive stages ( $0.28 \pm 0.09$ ) (Table 15), which was particularly visible in females of *P. polionotus* and *P. californicus* (Figure 10).

#### Preference index

As analyzed above, the preference index between the pup zone and the peanut butter zone is lower in parents than virgins. However, when experimental parents are compared to parents that were tested only once in a linear model (data untransformed), *Sex* and *Reproductive Stage* significantly influenced the preference index, and reduced the residual variation of the data (Table 14).

Overall, females had a higher preference index (Table 15). Similarly to the proportion of time spent in the pup zone, parents that were tested only once showed a higher preference index than experimental parents.

### *Infanticidal behaviours*

Experimental parents (n=32) never attacked an unfamiliar pup after they had had a litter for three days or more. At earlier reproductive stages (n=48), species appear to differ in their tendency to attack (Figure 11). Only few individuals attacked the unfamiliar pup in *P. maniculatus*, *P. leucopus* and *P. polionotus*, while at least a quarter of *P. californicus* males and a third of females showed an infanticidal behaviour at all stages before parenthood. In the three smaller species, no males but one *P. maniculatus* attacked the pup when they were expecting. On the other hand, the number of aggressive females did not show a decrease before parenthood. Individuals displaying an infanticidal behaviour as they were virgin or mated were often aggressive afterwards again (Figures 3-9, left hand side).

Most trials that had an aggressive outcome were immediately interrupted, which may explain why infanticidal individuals show very low values for parental behaviours (Figures 3-6, left hand side). Infanticidal individuals do not seem to differ from other individuals in their attraction/aversion-related behaviours (Figures 7-9, left hand side). The low number of attacks did not allow for a formal statistical analysis.

## **DISCUSSION**

Biparental care associated with monogamy has evolved at least twice independently in the wild mouse genus *Peromyscus* (Bedford and Hoekstra 2015). Previous laboratory and field studies have shown that *P. maniculatus* and *P. leucopus* are typically promiscuous with maternal care only, while *P. polionotus* and *P. californicus* independently developed biparental care associated with a monogamous mating system (Dewsbury 1981; Foltz 1981; Xia and Millar 1988; Ribble 1991; Wolff and Cicirello 1991; Jašarević et al. 2012; Bendesky et al. 2017). In this study, I further investigated these parental systems by measuring several parental behaviours and the attraction to pups of males and females of the above species at four reproductive stages, from sexual naivety to parenthood.

### *Parental behaviours*

Regardless of the measured behaviour, *P. maniculatus* was the least "parental" of all species under study. As expected, virgins, mated individuals, expecting individuals as well as fathers spent very little

time huddling and licking unfamiliar pups, built low quality nests and almost never retrieved the pups (Figures 3-6). Mothers of the same species displayed substantially more of parental behaviours, although this was not enough to match the median parental behaviours of the mothers of other species. In the wild, the home ranges of *P. maniculatus* males overlap with those of several females, and multiple inseminations are not uncommon (Birdsall and Nash 1973). As a result, paternity certainty is low and males may benefit from not caring for pups and seeking new mating opportunities.

*P. leucopus* showed similar trends for the time spent huddling and licking pups (Figures 3-4). However and surprisingly, the nest quality and proportion of times the pup was retrieved were intermediate at all reproductive stages (Figures 5-6). This trend was also visible in males (to a lesser extent), which aligns with previous laboratory-based studies, but contradicts two studies done in a more natural setting, where males provided virtually no care to their litter and were aggressively excluded from the nest by their mate (Xia and Millar 1988 and citations therein; Wolff and Cicirello 1991). Xia and Millar argued that the paternal behaviours observed in captive *P. leucopus* may be a laboratory artifact resulting from phenotypic plasticity, and may provide little to no fitness advantage in the wild (Xia and Millar 1988). In my study, paternal behaviours may thus result from the prolonged exposure of males to pups and their inability to desert the breeding cage. Alternatively, there may be some intra-specific variation in parental behaviours, since the subspecies used by Xia and Miller and Wolff and Cicirello (*P. leucopus noveboracensis*) is different from the one used here (Xia and Millar 1988; Wolff and Cicirello 1991).

In contrast with the promiscuous species, *P. polionotus* showed relatively high levels of parental behaviours at all reproductive stages, especially in females. Mothers were the most parental across all behaviours except for the time spent licking, which was higher at earlier stages (Figure 4). The latter observation was already made in this colony (Bendesky et al. 2017, Extended Data Fig. 5a), and highlights the fact that licking pups is not restricted to parents. If we consider that mice have a restricted time to care for pups, it could be that mothers invest more time in huddling over the pup while non-mother females may spend their time investigating and licking the unfamiliar pup (personal observation).

In theory, adults that have not had their own litter should only care for pups that are not their own when the benefit of caring is higher than the benefit of dispersing and trying to reproduce. Foltz repeatedly found young *P. polionotus* adults in their natal nest, alongside with their parents and the next litter of dependent young (Foltz 1981). As it is highlighted by the work of Margulis and co-workers (Margulis et al. 2004), virgin *P. polionotus* females that remain in the natal nest through the rearing of a younger litter of siblings become better mothers (wean a significantly higher number of pups) than females that were never exposed to pups. Since this species is monogamous, two consecutive litters usually consist of full siblings (Foltz 1981). If individuals of a first litter participate in the care of their young siblings instead of breeding themselves, they may benefit from it through inclusive fitness and through the gain of rearing experience. Indeed, displaying parental behaviours before being a parent may be adaptive in this short-lived monogamous species, as the experience gained through the rearing of siblings may increase the chance of a successful first litter and positively affect an individual's lifetime reproductive success (Margulis et al. 2004).

As opposed to *P. polionotus*, male and female *P. californicus* showed equivalent amounts of parental care. Both sexes spent little time huddling and licking the unfamiliar pup, built low-quality nests and retrieved the pup few times until their own litter was born. They then increased their parental behaviours substantially (Figures 3-6). While most individuals transitioned abruptly from being non-parental to taking care of an unfamiliar pup, the variation in the amount of parental care increased over reproductive stages. Individual variation in the onset of parental behaviour has already been investigated in detail in male *P. californicus* (Gubernick et al. 1994), and it has been suggested that their social environment before and after mating can have a strong impact on their parental behaviour across reproductive stages. Additionally, an individual's dominance status as a virgin (and within the pair) and the amount of parental care received as a juvenile could influence parental behaviours greatly (Brown 1993; Gleason and Marler 2013). The latter factors were not controlled for in the present study and may represent an additional source of variation.

One may wonder why the onset of parental care mostly happens at parenthood in *P. californicus*, while it seems to occur earlier in *P. polionotus*. A possible explanation lies in the differences in dispersal and territoriality of these species. Two studies on the dispersal of *P. polionotus ammobates*

described the dispersal of subadults as short movements away from their natal home range (Lynn 2000; Swilling and Wooten 2002). As a result, the home ranges of adults tend to overlap within family groups (Lynn 2000; Swilling and Wooten 2002). Furthermore, although males were found to sire at least two to three consecutive litters with the same partner, females appear to change mates relatively often, with the new mate occupying the same burrow as the female and her litter (Foltz 1981). If we make the assumption that the above characteristics hold true for the subspecies of interest, then it stands to reason that adult mice should tolerate or care for the pups they encounter before parenthood, as they are likely to be highly related to them.

In contrast, *P. californicus* is a strongly territorial species, and the home ranges of adjacent adults are largely exclusive and statistically distinguishable (Ribble and Salvioni 1990). Moreover, there does not seem to be any extra-pair mating in this species (Ribble 1991). The two above characteristics ensure that any pup found within an adult's home range that is not its own (obvious when the adult is unmated or recently mated) is that of an invading pair. As a result, an adult *P. californicus* who has never had pups would not necessarily benefit from caring or tolerating unfamiliar pups. This idea is supported by the relatively high rate of infanticidal attacks observed here (Figure 11), and reported elsewhere (Gubernick et al. 1994).

This difference in the onset of parental care between two biparental species of the same genus suggests a difference in the proximate mechanisms underlying biparental care and monogamy, and highlights the independent evolution of these traits.

#### *Attraction/aversion behaviours*

Counter to my expectations, the latency of physical contact after first approach did not relate to the data from the test of attraction/aversion to a pup. *P. maniculatus* seemed scared of the pup and rarely approached/made physical contact with it before parenthood (personal observation; Figure 7). Across all species, mothers were very quick to make physical contact with the pup when compared to other mice, which may demonstrate their motivation to care for the pup.

In the test of attraction/aversion to a pup, however, experimental mice did not seem more attracted to pups as parents than at earlier stages (Figures 8-9). Surprisingly, virgins were significantly more

attracted to the pup than other stages (seen for both attraction variables: proportion of time spent in the pup zone and preference index). These results could either mean that (1) virgins are generally more attracted to pups than paired individuals in that test or that (2) repeating the test four times on the same individuals led to them being habituated and not interested in a pup that they know they cannot reach. To investigate the latter idea, I used the attraction/aversion data from other parent mice (parents only) of three species (all but *P. maniculatus*) and compared them to those of the experimental parents (Figure 10). Parents only were significantly more attracted to the pup than experimental parents in both the proportion of time spent close to the pup and the preference index. This suggests that animals tested four times became habituated and were not as interested in the pup as animals discovering the arena for the first time. To further test this hypothesis, independent measurements should be done on mice at different reproductive stages and compared to the repeated measurements done in this study. Habituation may also have had an impact on the parental behaviour data, but its effect was not explored here.

#### *Infanticide and the onset of parental behaviours*

The proportion of individuals attacking the unfamiliar pup was relatively low in all of the smaller species when compared to *P. californicus* (Figure 11). Interestingly, parents never attacked the unfamiliar pup, which gives further support to the idea that parent rodents tend not to actively discriminate between their offspring and unfamiliar young within their home range (e.g. Elwood 1977; Cicirello and Wolff 1990; Wolff and Cicirello 1991).

Overall, females did not become less aggressive across reproductive stages before they gave birth to their own litter. In contrast, none of the males of smaller species except one *P. maniculatus* behaved infanticidally when expecting a litter (Figure 11). This is comparable to field-based studies done on *Peromyscus* and *Mus*, where (1) female mice killed most unfamiliar pups found outside of their nest until parturition, and (2) dispersing and unmated males were usually infanticidal, while mated males did not kill pups within their home range (McCarthy and vom Saal 1985; Soroker and Terkel 1988; Wolff and Cicirello 1991).

Males and females are likely to differ in the precision of information they get concerning the timing of birth of their young. This is especially true in internal fertilizers like mammals, where fertilization is followed by an extended period of internal embryonic development (Elwood 1994). Physiological and hormonal changes during pregnancy and parturition provide females with precise information about their own litter's birth, and females may thus behave infanticidally until parturition to prevent older, unfamiliar pups from competing for resources with their future litter (Wolff and Cicirello 1991; Elwood 1994; Dulac et al. 2014). Males, however, may only have an approximate idea of the time at which their mate will give birth, and could therefore benefit from an early inhibition of infanticidal behaviours (Elwood 1994; Gubernick et al. 1994). For example, in the house mouse (*Mus musculus*), females behave infanticidally until parturition and again soon after weaning in the wild (McCarthy and vom Saal 1985). Males, however, rely on a timed neural mechanism triggered by coital ejaculation that inhibits infanticidal behaviour and launches the onset of paternal care some days before their mate gives birth (Elwood 1985; Soroker and Terkel 1988; Perrigo et al. 1990; Perrigo et al. 1991). Similarly, female Mongolian gerbils and dwarf hamsters tend to be infanticidal until very late pregnancy, while males progressively lose their infanticidal behaviour as their mate's pregnancy is coming to an end (Elwood 1977; Vella et al. 2005). This sex difference may be even stronger in the case of species where males mate with several females and do not form long-term associations with them. These males are unlikely to receive precise information on the advancement of a female's pregnancy and should therefore stop behaving infanticidally well before the birth of their young, to avoid the risk of harming them by mistake. Monogamous males, however, are likely to obtain more precise information about their mate's gestation through chemical cues in the female's urine or produced during parturition (Gubernick and Alberts 1989; Brown 1993), and may stop behaving infanticidally later in pregnancy.

A transition from infanticidal or non-caring behaviours to parental behaviours when young are expected has been observed in many rodents, but also in a number of species across the animal kingdom, from arthropods to humans (Elwood and Mason 1993; Elwood 1994; Dulac et al. 2014). The inhibition of infanticide and the onset of parental behaviour do not necessarily align with the time of birth of an animal's own offspring, but may occur earlier in its life, depending on the species'

fertilization mode and mating system, and on the animal's sex, reproductive status and previous social experience (Elwood 1985; Brown 1993; Elwood 1994; Gubernick et al. 1994; Dulac et al. 2014). In the present study, I show that four murid species with different mating systems differ in their parental behaviour across sexes and reproductive stages.



## LITERATURE CITED

- Bates D, Maechler M, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. doi: 10.18637/jss.v067.i01
- Bedford NL, Hoekstra HE (2015) *Peromyscus* mice as a model for studying natural variation. *Elife* 4:1–13. doi: 10.7554/eLife.06813
- Bendesky A, Kwon Y-M, Lassance J-M, et al (2017) The genetic basis of parental care evolution in monogamous mice. *Nature* 544:434–439.
- Birdsall D, Nash D (1973) Occurrence of multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution* (N Y) 27:106–110.
- Brown RE (1993) Hormonal and experiential factors influencing parental behaviour in male rodents : An integrative approach. *Behav Processes* 30:1–28.
- Cicirello DM, Wolff JO (1990) The effects of mating on infanticide and pup discrimination in white-footed mice. *Behav Ecol Sociobiol* 26:275–279.
- Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press
- Dewsbury D (1981) An exercise in the prediction of monogamy in the field from laboratory data on 42 species of Muroid rodents. *Biologist* 63:138–162.
- Dulac C, O’Connell LA, Wu Z (2014) Neural control of maternal and paternal behaviors. *Science* (80-) 345:1063–1069.
- Elwood R, Mason C (1993) The couvade and the onset of paternal care in humans: a biological perspective. *Behav Processes* 28:226–227. doi: 10.1016/0376-6357(93)90102-W
- Elwood RW (1977) Changes in the responses of male and female gerbils (*Meriones unguiculatus*) towards test pups during the pregnancy of the female. *Anim Behav* 25:46–51. doi: 10.1016/0003-

- Elwood RW (1994) Temporal-based kinship recognition: A switch in time saves mine. *Behav Processes* 33:15–24. doi: 10.1016/0376-6357(94)90057-4
- Elwood RW (1985) Inhibition of infanticide and onset of paternal care in male mice (*Mus musculus*). *J Comp Psychol* 99:457–467. doi: 10.1037/0735-7036.99.4.457
- Foltz DW (1981) Genetic Evidence for Long-Term Monogamy in a Small Rodent, *Peromyscus polionotus*. *Am Nat* 117:665–675.
- Gleason ED, Marler C a (2013) Non-genomic transmission of paternal behaviour between fathers and sons in the monogamous and biparental California mouse Non-genomic transmission of paternal behaviour between fathers and sons in the monogamous and biparental California mouse. *Proc R Soc B Biol Sci* 280:20130824. doi: 10.1098/rspb.2013.0824
- Gubernick DJ, Alberts JR (1989) Postpartum maintenance of paternal behaviour in the biparental California mouse, *Peromyscus californicus*. *Anim Behav* 37:656–664.
- Gubernick DJ, Schneider KA, Jeannotte LA (1994) Individual differences in the mechanisms underlying the onset and maintenance of paternal behavior and the inhibition of infanticide in the monogamous biparental California mouse, *Peromyscus californicus*. *Behav Ecol Sociobiol* 34:225–231.
- Gubernick DJ, Teferit T (2000) Adaptive significance of male parental care in a monogamous mammal. *Proc Biol Sci* 267:147–150.
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical J* 50:346–363.
- Jašarević E, Bailey DH, Crossland JP, et al (2012) Evolution of monogamy, paternal investment, and female life history in *Peromyscus*. *J Comp Psychol* 127:91–102. doi: 10.1037/a0027936

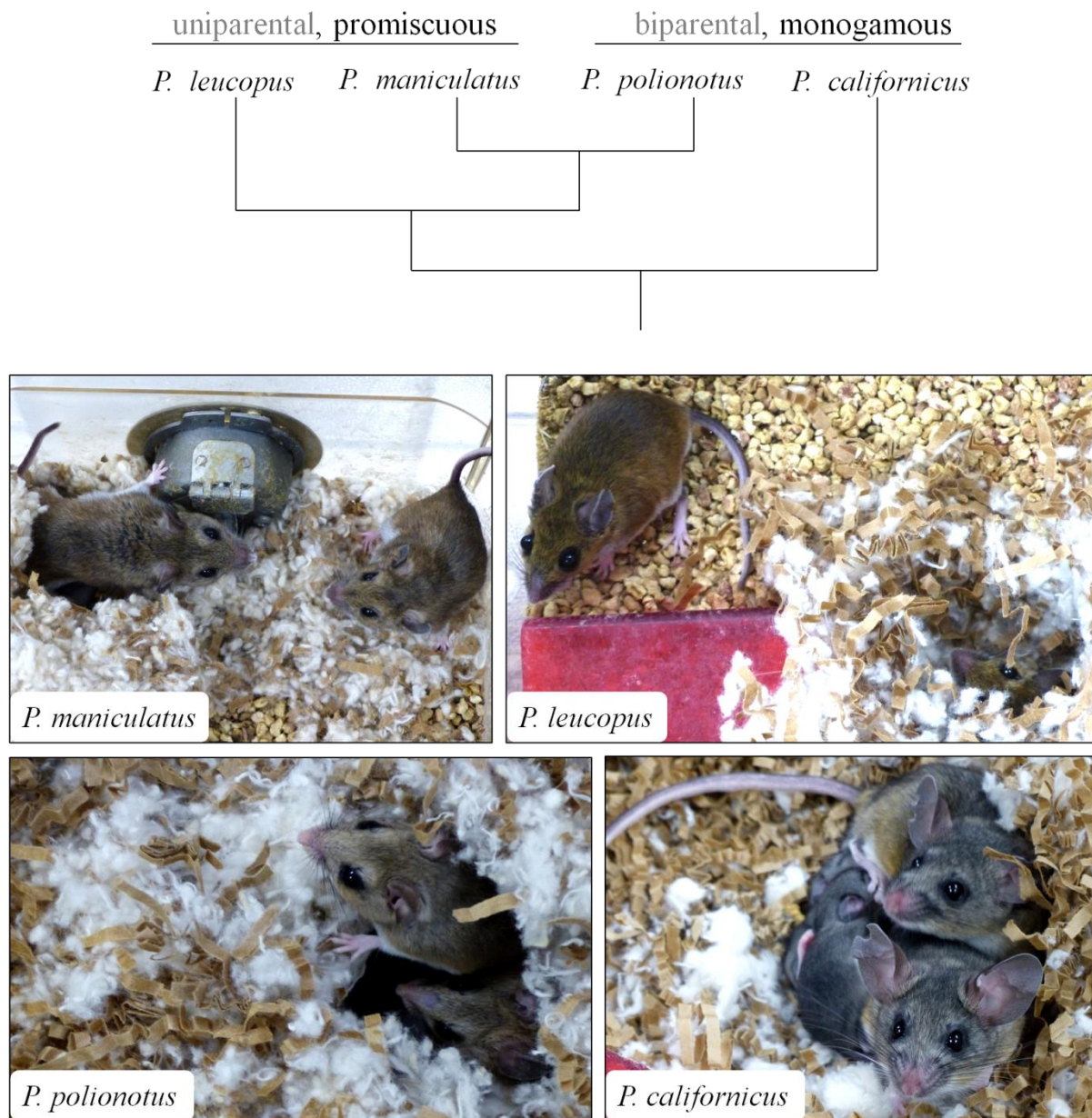
- Kleiman DG (1977) Monogamy in mammals. *Q Rev Biol* 52:39–69.
- Kleiman DG, Malcolm J (1981) The evolution of male parental investment. In: Gubernick DJ, Klopfer P (eds) *Parental care in mammals*. Plenum, New York, pp 347–387
- Lonstein JS, De Vries GJ (2000) Sex differences in the parental behavior of rodents. *Neurosci Biobehav Rev* 24:669–686.
- Lynn W (2000) Social organization and burrow site selection of the Alabama beach mouse (*Peromyscus polionotus ammobates*). Auburn University, Alabama
- Margulis SW (1998) Relationships among parental inbreeding, parental behaviour and offspring viability in oldfield mice. *Anim Behav* 55:427–438.
- Margulis SW, Nabong M, Alaks G, et al (2004) Effects of early experience on subsequent parental behaviour and reproductive success in oldfield mice, *Peromyscus polionotus*. *Anim Behav* 69:627–634. doi: 10.1016/j.anbehav.2004.04.021
- McCarthy MM, vom Saal FS (1985) The influence of reproductive state on infanticide by wild female house mice (*Mus musculus*). *Physiol Behav* 35:843–849.
- Noldus LPJJ, Spink AJ, H RAJT (2001) EthoVision : A versatile video tracking system for automation of behavioral experiments. *Behav Res Instruments, Methods Comput* 33:398–414.
- Perrigo G, Belvin L, vom Saal FS (1991) Individual variation in the neural timing of infanticide and parental behavior in male house mice. *Physiol Behav* 50:287–96. doi: 10.1016/0031-9384(91)90068-Y
- Perrigo G, Bryant WC, vom Saal FS (1990) A unique neural timing system prevents male mice from harming their own offspring. *Anim Behav* 39:535–539. doi: 10.1016/S0003-3472(05)80419-1
- RCoreTeam (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- Ribble D (1991) The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav Ecol Sociobiol* 29:161–166.
- Ribble DO, Salvioni M (1990) Social organization and nest co-occupancy in *Peromyscus californicus*, a monogamous rodent. *Behav Ecol Sociobiol* 26:9–15.
- Soroker V, Terkel J (1988) Changes in incidence of infanticidal and parental responses during the reproductive cycle in male and female wild mice *Mus musculus*. *Anim Behav* 36:1275–1281.
- Stockley P, Hobson L (2016) Paternal care and litter size coevolution in mammals. *Proc R Soc B Biol Sci* 283:20160140. doi: 10.1098/rspb.2016.0140
- Swilling WR, Wooten MC (2002) Subadult dispersal in a monogamous species: the Alabama beach mouse (*Peromyscus polionotus ammobates*). *J Mammal* 83:252–259.
- Vella ET, Evans CCD, Ng MWS, Wynne-Edwards KE (2005) Ontogeny of the transition from killer to caregiver in dwarf hamsters (*Phodopus campbelli*) with biparental care. *Dev Psychobiol* 46:75–85. doi: 10.1002/dev.20047
- Wolff JO (1989) Social behavior. In: Kirkland G, Layne N (eds) *Advances in the study of Peromyscus* (Rodentia), 1st edn. Texas Tech University Press, p 366
- Wolff JO, Cicirello DM (1991) Comparative paternal and infanticidal behavior of sympatric white-footed mice (*Peromyscus leucopus noveboracensis*) and deermice (*P. maniculatus nubiterrae*). *Behav Ecol* 2:38–45.
- Wolff JO, Lundy K, Baccus R (1988) Dispersal, inbreeding avoidance and reproductive success in white-footed mice. *Anim Behav* 36:456–465.
- Xia X, Millar JS (1988) Paternal behavior by *Peromyscus leucopus* in enclosures. *Can J Zool* 66:1184–1187.

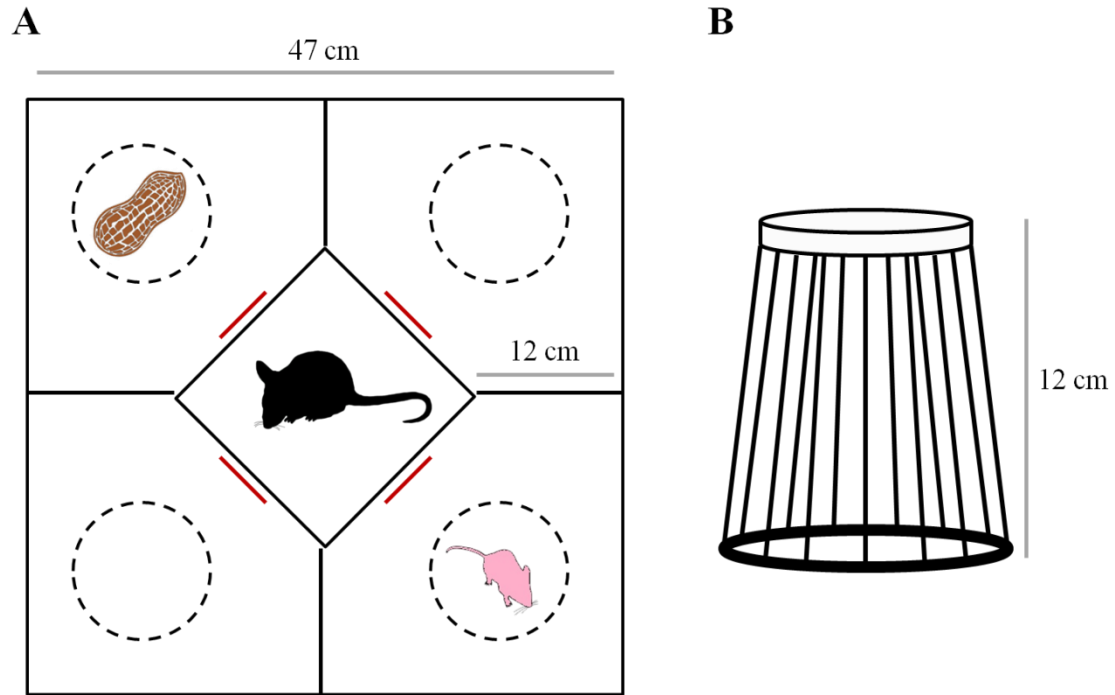
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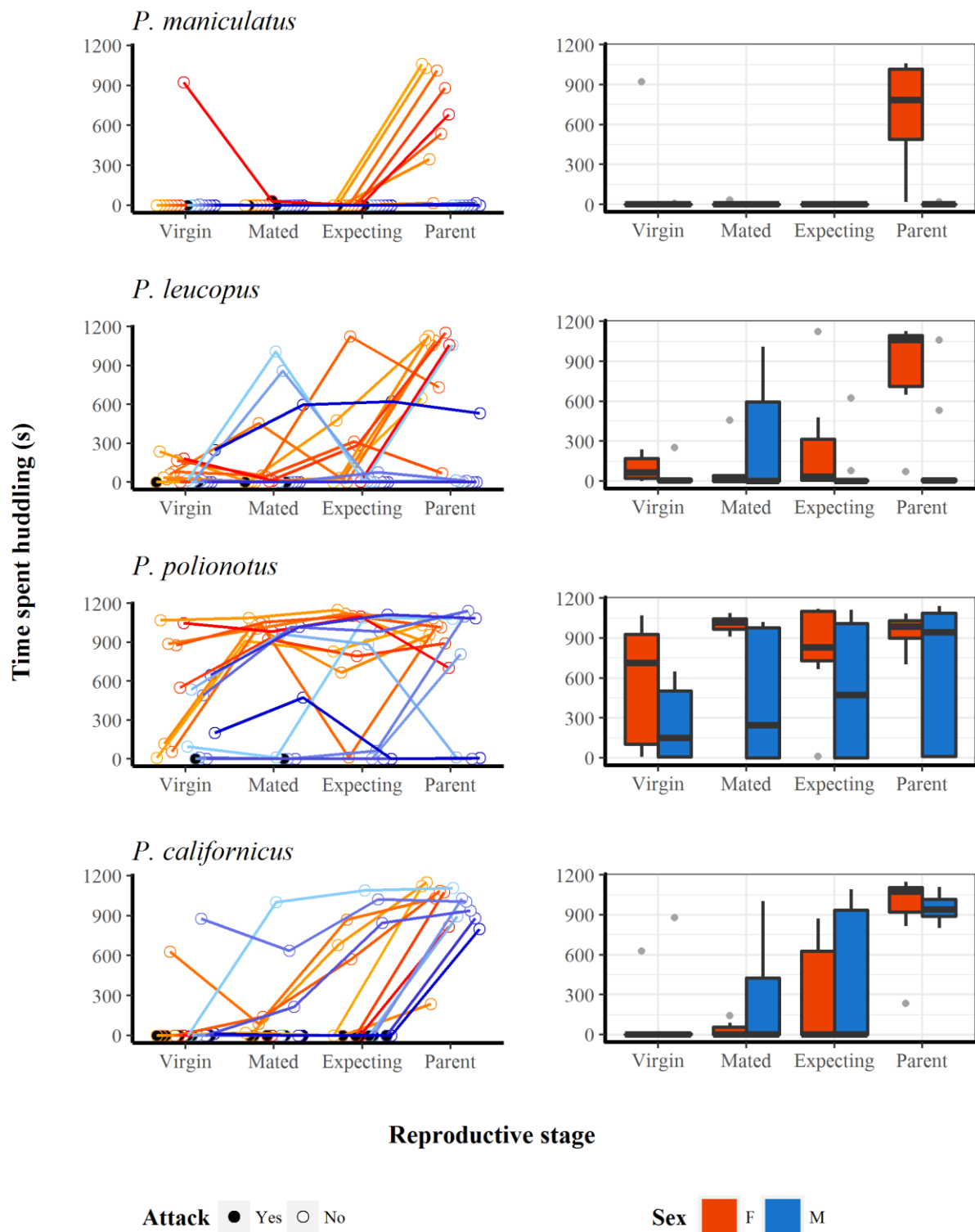
## FIGURES AND TABLES



**Figure 1 - Monogamy and biparental care have evolved at least twice independently in the wild mouse genus *Peromyscus*.** Phylogenetic relationships between the species under study (not at scale, adapted from Bedford and Hoekstra (2015)) and representative pictures of each species. *P. maniculatus* and *P. leucopus* breeding pairs (upper pictures), typically with the female inside the nest and the male outside. *P. polionotus* and *P. californicus* breeding pairs (lower pictures), typically with both parents inside the nest.

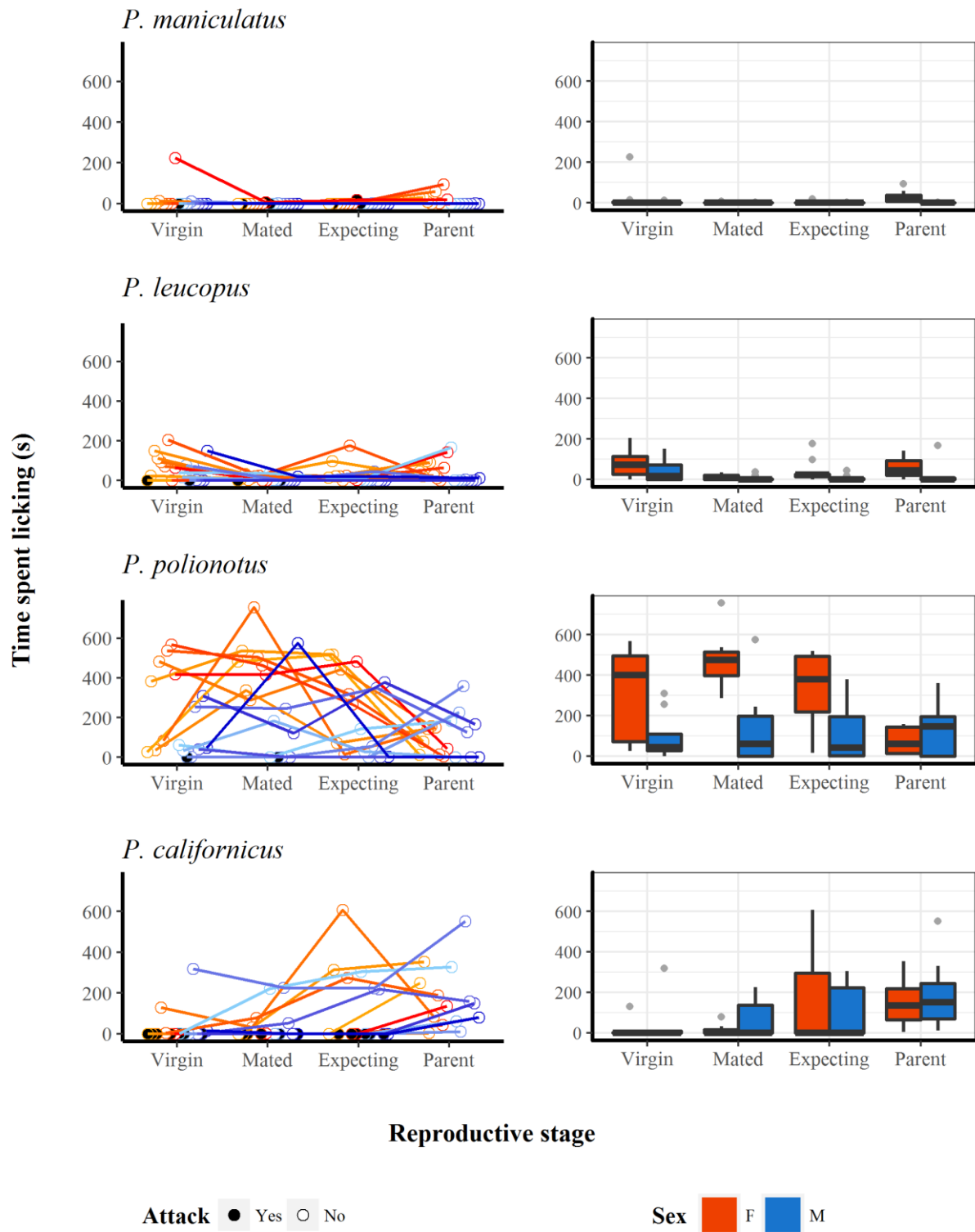


**Figure 2 - Illustration of the experimental material for the test of attraction/aversion to pup.** (A) Top view of the experimental arena (not to scale). In the top left corner, the peanut represents the peanut butter. In the bottom right corner, the smaller pink mouse silhouette represents an unfamiliar pup (locations among peripheral compartments were randomized). In the centre, the larger black mouse silhouette represents the test individual, who was allowed to travel between compartments through the four open doors, represented in red. Pencil holders are contoured with a dashed line. (B) Schematic pencil holder, with a wall made of metallic bars that allow air and sound to travel freely.

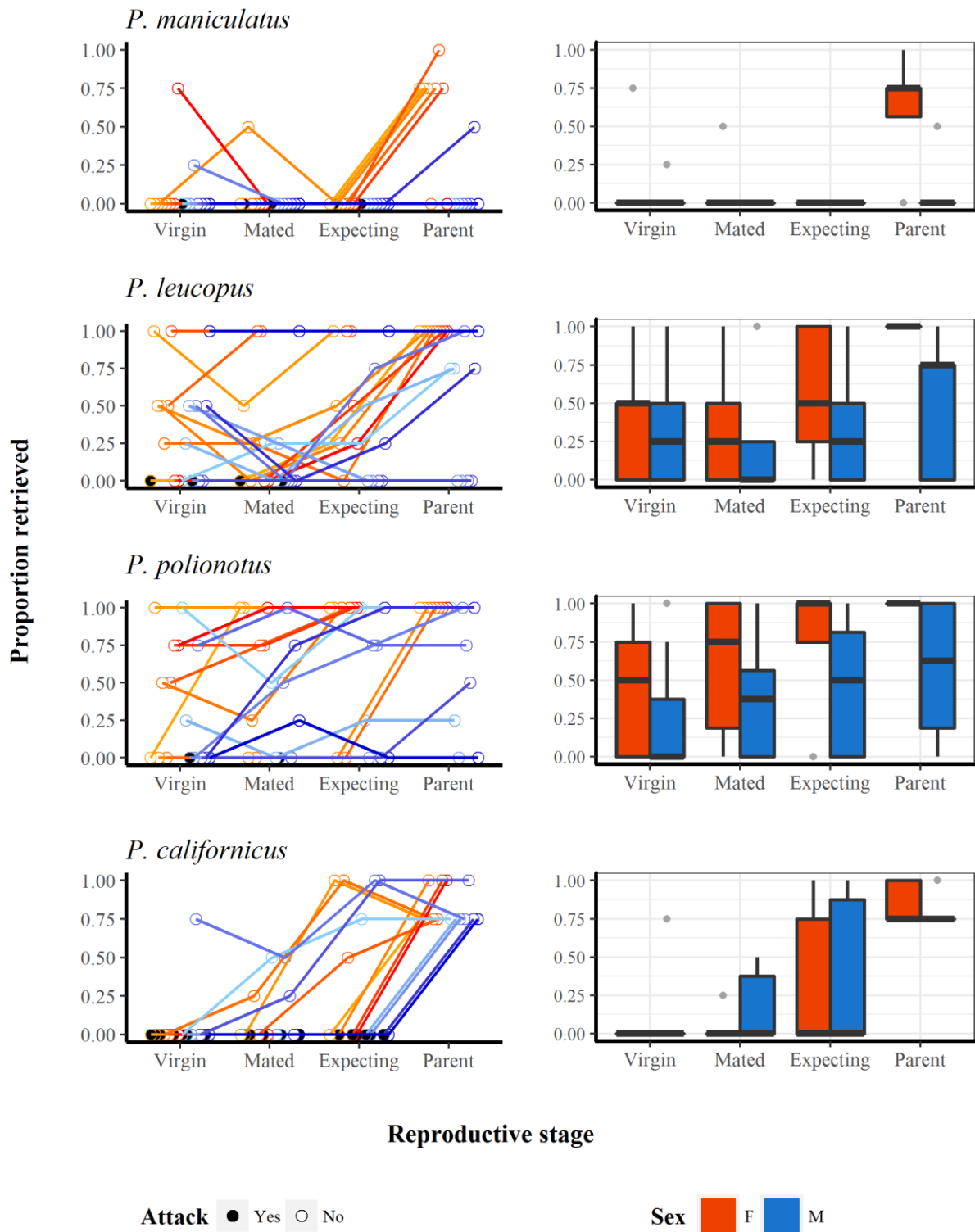


**Figure 3 - Time spent huddling for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.

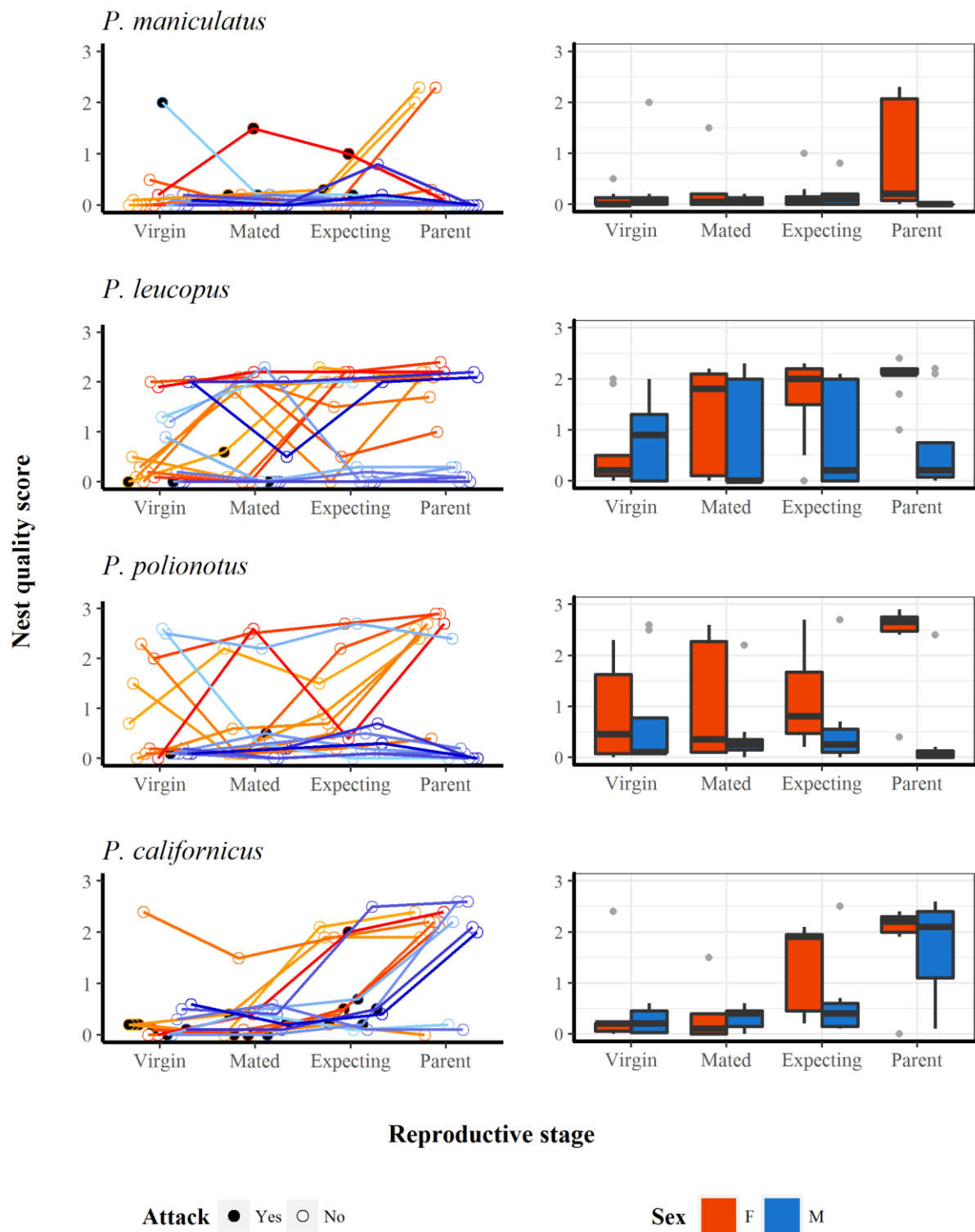




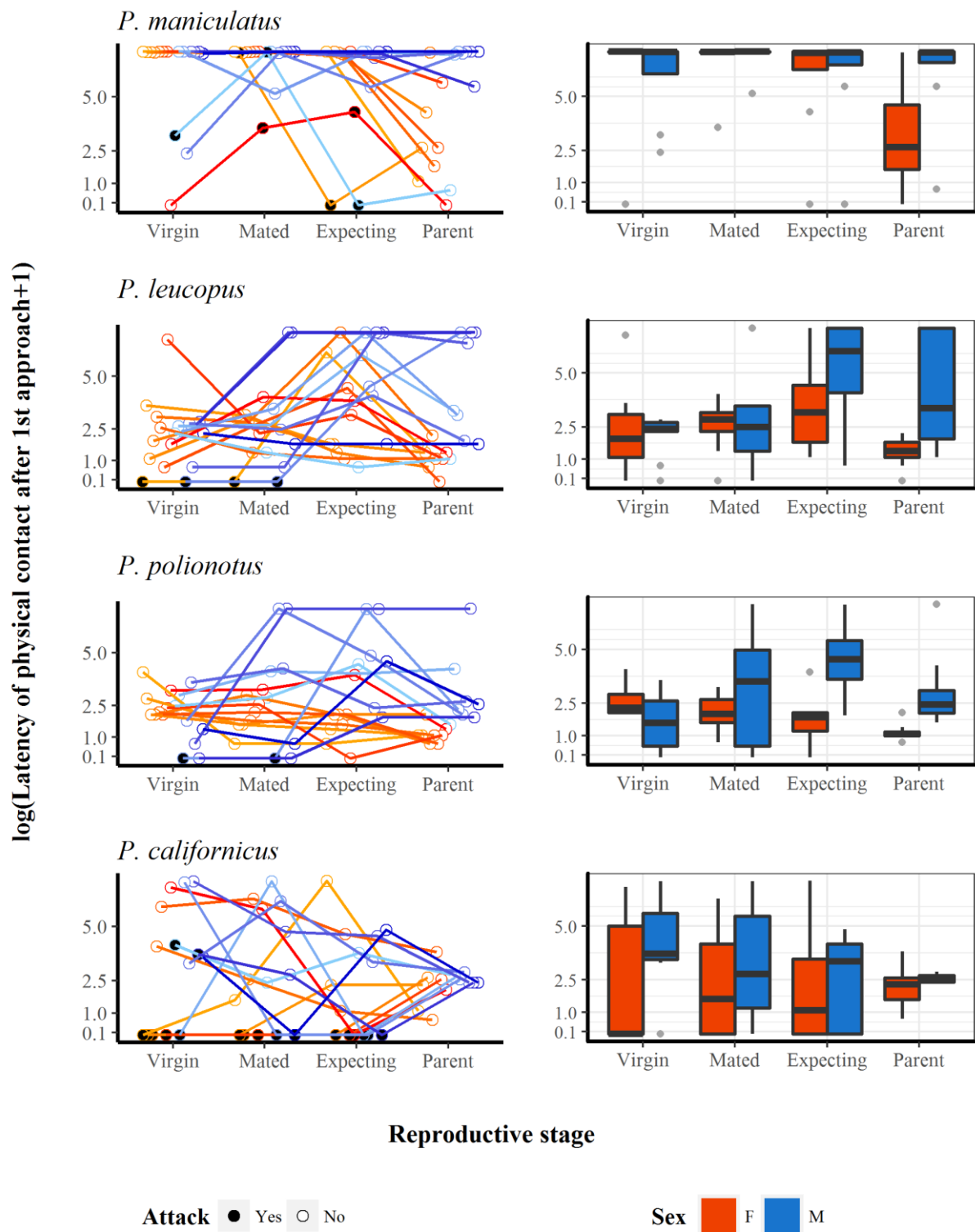
**Figure 4 - Time spent licking for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.



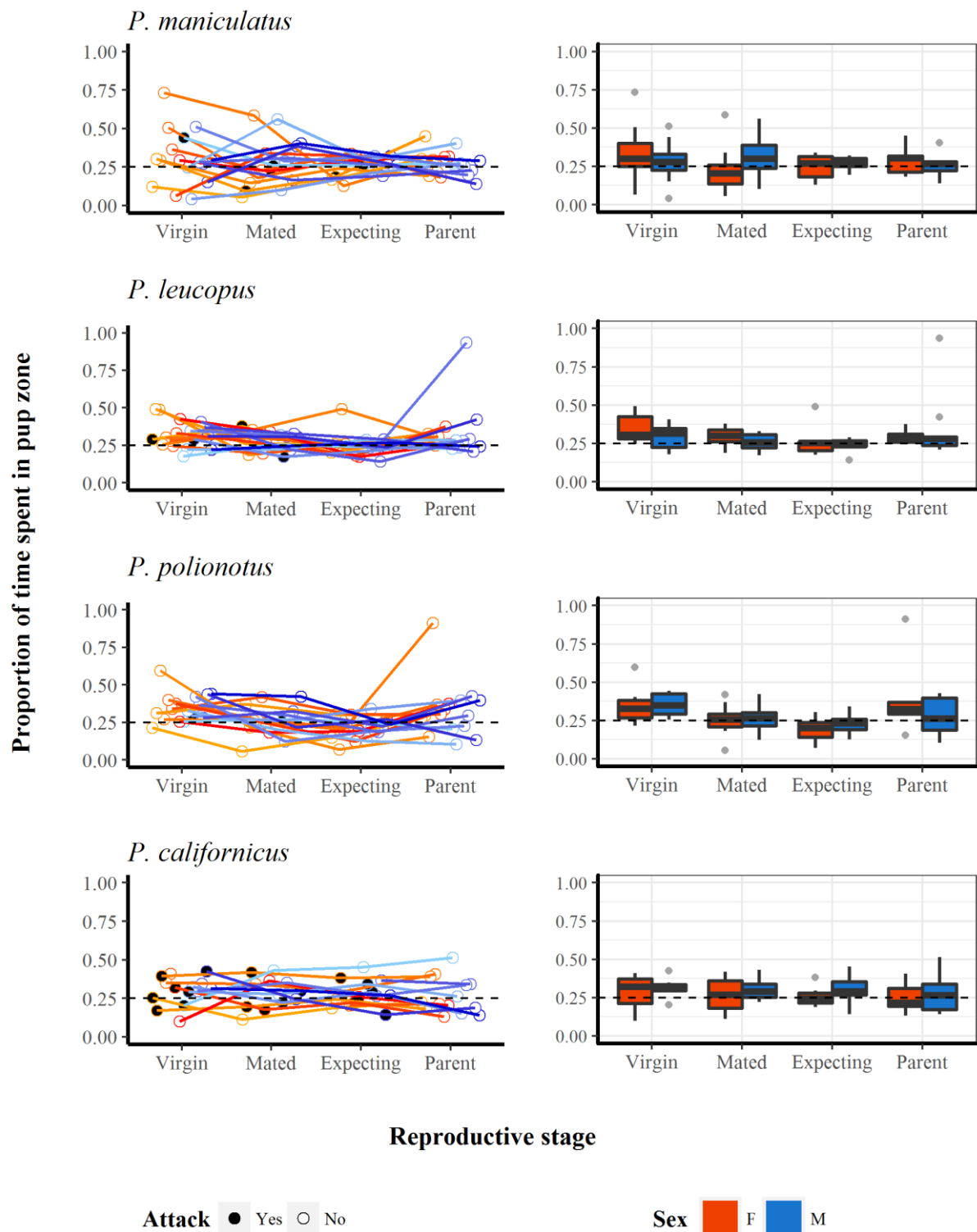
**Figure 5 - Proportion of times the pup was retrieved for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.



**Figure 6 - Nest quality score at 50' for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.

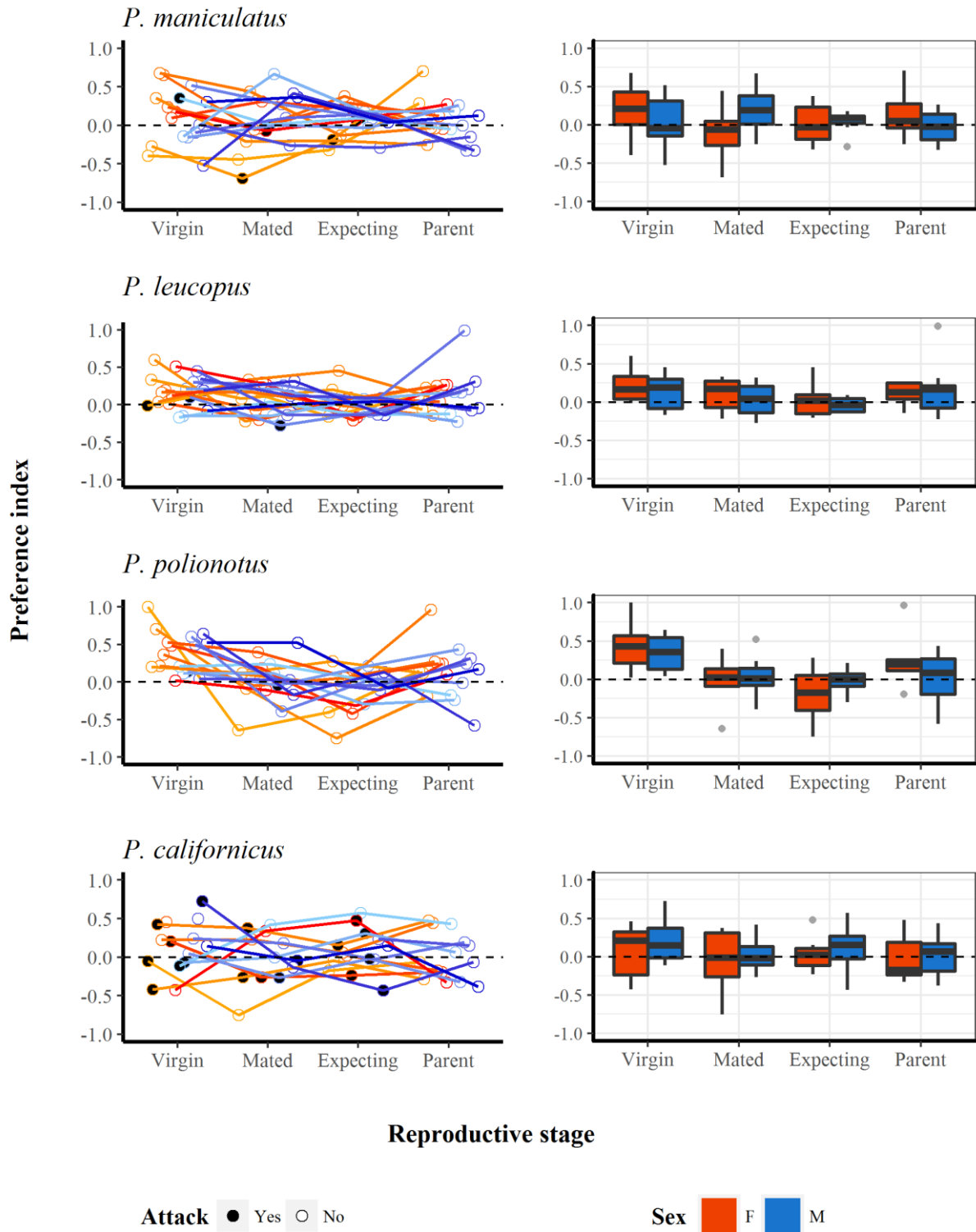


**Figure 7 - Latency of physical contact after first approach for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), with a log scale on the y axis. Females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.

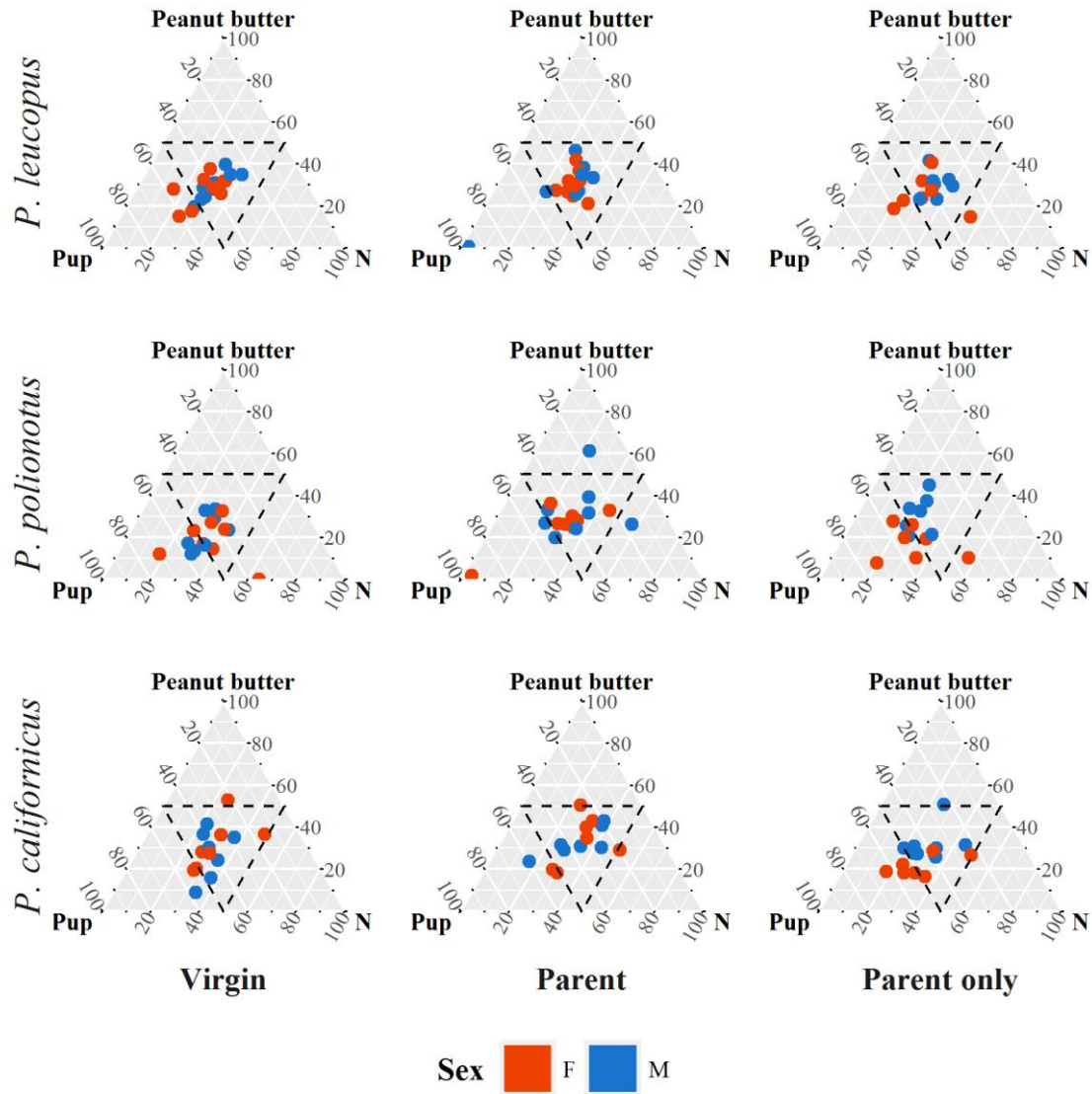


**Figure 8 - Proportion of time spent in the pup zone for all species, at all reproductive stages for both sexes.**

The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. The dashed line is the expected proportion if no preference is shown. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.

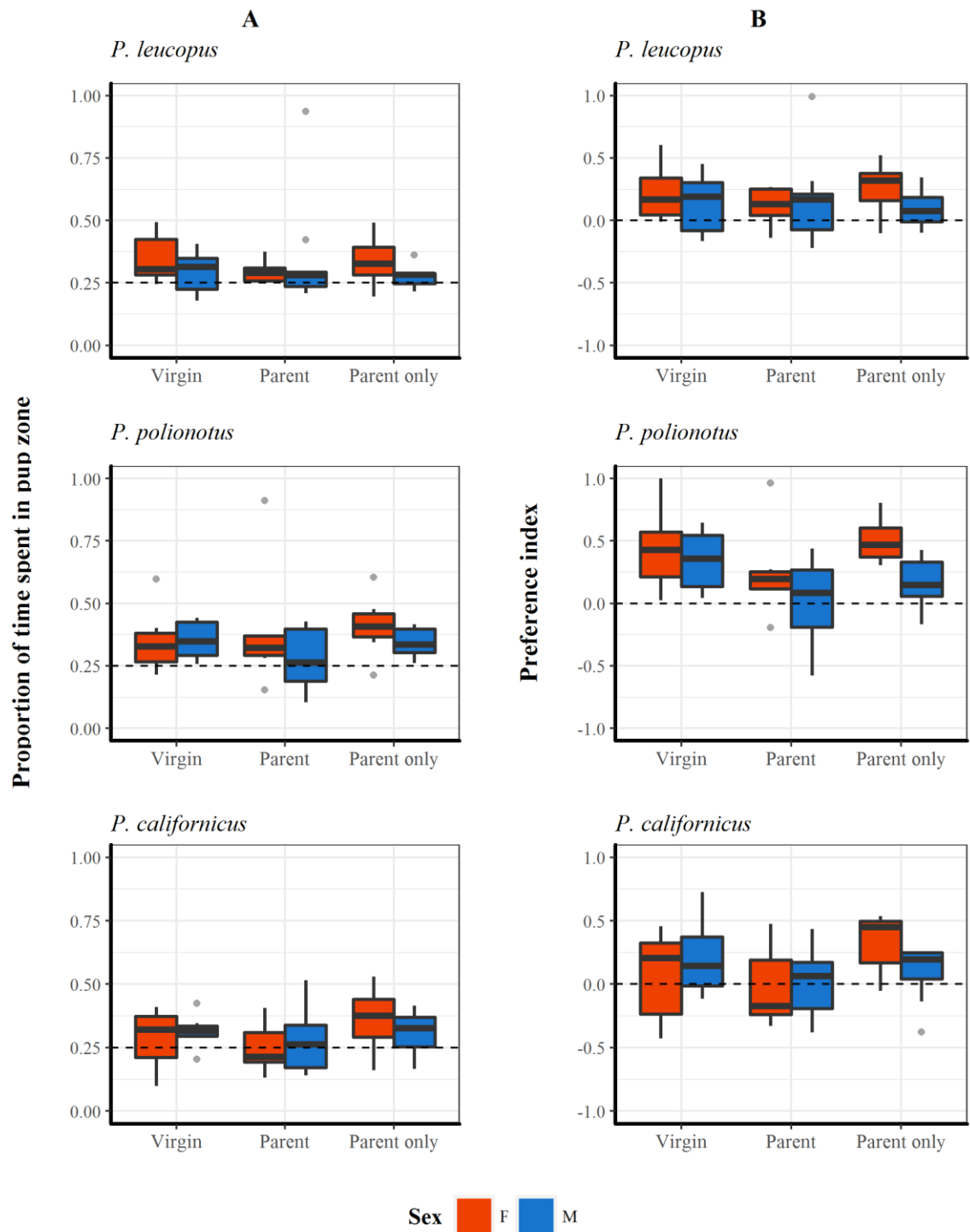


**Figure 9 - Preference index for all species, at all reproductive stages for both sexes.** The data are represented as individual trajectories (lineplots, left hand side) and as group trends (boxplots, right hand side), where females are in warm colours and males in cold colours. Trials that resulted in an attack are marked in black on the lineplots. The dashed line is the expected index if no preference is shown. Sample sizes: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.



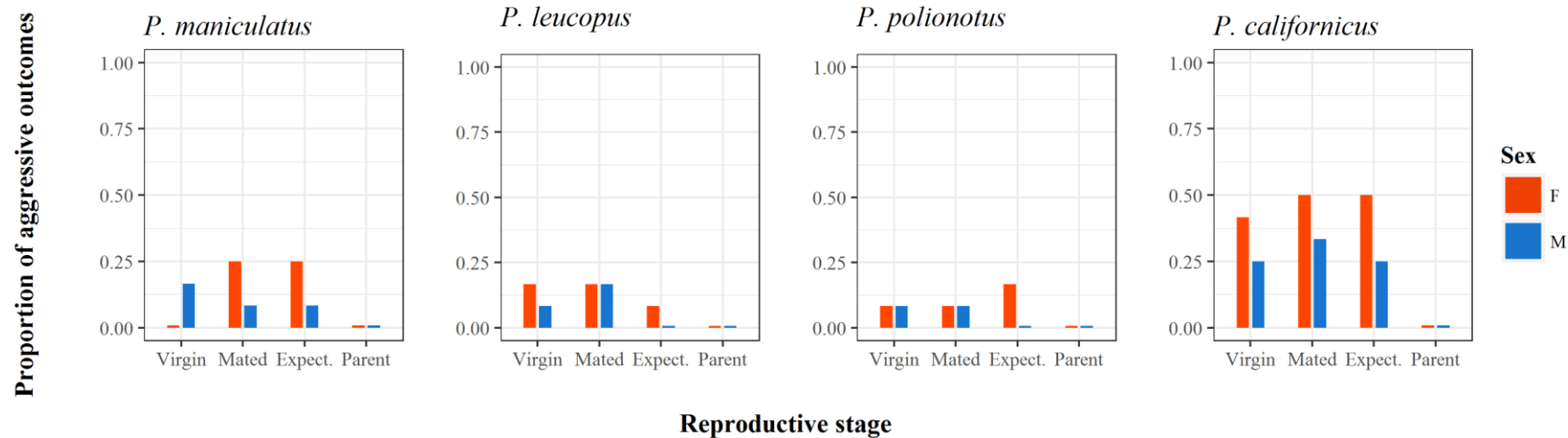
**Figure 10 - Ternary plots for the test of attraction/aversion to a pup for the same individuals as virgins and parents (left two panels), and for other individuals tested only as parents (right panels).** Each data point is placed according to three coordinates, depending on the proportion of time spent in the pup zone, ("Pup"), the peanut butter zone ("Peanut butter") and the zones containing empty holders ("N"). N represents the average time spent in the two zones containing empty holders. The dashed lines are set at 50% for each axis. Sample sizes: *P. leucopus*, n=9 pairs for the first two panels, n=7 pairs for the third panel; *P. polionotus*: n=8 pairs for the first two panels, n=7 pairs for the third panel; *P. californicus*: n=7 pairs for the first two panels, n=8 pairs for the third panel.





**Figure 11 - Attraction/aversion test results for experimental mice as virgins and parents, and for other individuals tested only as parents.** (A) Proportion of time spent in the pup zone. The dashed line is the expected proportion if no preference is shown (B) Preference index. The dashed line is the expected index if no preference is shown. Sample sizes for (A) and (B): *P. leucopus*: n=9 pairs (virgin and parent), n=7 (parent only); *P. polionotus*: n=8 pairs (virgin and parent), n=7 pairs for the third panel; *P. californicus*: n=7 pairs (virgin and parent), n=8 pairs (parent only).





1

2 **Figure 12 - Proportion of individuals who attacked the unfamiliar pup, for all species when experimental mice were virgin, mated or expecting a litter.** No parent in  
 3 any species was infanticidal. Since no formal analysis was performed on these data, all experimental pairs are included as virgin, mated and expecting mice (n=12 pairs for  
 4 each species). Sample sizes as parents: *P. maniculatus*: n=8 pairs, *P. leucopus*: n=9 pairs, *P. polionotus*: n=8 pairs, *P. californicus*: n=7 pairs.

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7 **Table 1 - Significance of fixed effect factors on behaviours measured in the Parental behaviour test.**  $\chi^2$ ,  $p$  values and the Akaike Inference Criteria (AIC) are given by  
8 the *anova* function. As at least one two-way interaction term had a significant effect on the measured behaviour in all instances, the significance of main effects (*Species*, *Sex*  
9 and *Reproductive Stage*) was not tested.

Fixed effect	Time spent huddling			Time spent licking			Proportion retrieved			Nest quality score		
	$\chi^2$	$p$	$\Delta AIC$	$\chi^2$	$p$	$\Delta AIC$	$\chi^2$	$p$	$\Delta AIC$	$\chi^2$	$p$	$\Delta AIC$
<i>Species : Sex</i>	6.625	0.085	-10.0	7.695	0.053	1.6	4.660	0.199	-1.3	3.387	0.336	-2.6
<i>Species : Reprod. Stage</i>	36.547	3.169E-05***	18.6	47.799	2.785E-07***	28.2	32.610	1.561E-04***	14.6	16.245	0.062	1.8
<i>Sex : Reprod. Stage</i>	19.416	2.242E-04***	13.4	1.444	0.695	-4.5	22.367	5.472E-05***	16.4	26.419	7.794E-06***	20.4

12 **Table 2 - Model summary for parental behaviours, after removal of non-significant interaction term(s).** Estimates, standard errors (SE) and z values are extracted using  
13 the *summary* function. Estimates are given relative to the reference: *P. maniculatus* female virgin.

Fixed effect levels	Time spent huddling			Time spent licking			Proportion retrieved			Nest score		
	Estimate	SE	z value	Estimate	SE	z value	Estimate	SE	z value	Estimate	SE	z value
(Intercept)	1.057	0.636	1.661	1.212	0.487	2.488	0.098	0.090	1.086	-2.009	0.251	-8.017
<i>P. leucopus</i>	2.003	0.782	2.561	2.266	0.630	3.596	0.299	0.111	2.702	1.158	0.261	4.432
<i>P. polionotus</i>	4.192	0.805	5.210	3.699	0.649	5.703	0.281	0.114	2.473	1.040	0.269	3.872
<i>P. californicus</i>	0.564	0.833	0.677	0.483	0.671	0.719	-0.009	0.118	-0.076	1.031	0.279	3.702
Male	-0.985	0.569	-1.731	-1.086	0.327	-3.324	-0.070	0.080	-0.874	0.096	0.269	0.358
Mated	-0.502	0.703	-0.715	-0.478	0.525	-0.910	-0.035	0.092	-0.380	0.290	0.217	1.337
Expecting	-0.493	0.703	-0.702	-0.412	0.525	-0.785	0.434	0.092	4.688	0.866	0.217	3.987
Parent	3.760	0.703	5.351	0.805	0.525	1.533	-0.016	0.083	-0.189	1.489	0.217	6.856
Male : Mated	0.317	0.629	0.505	-	-	-	-0.055	0.083	-0.661	-0.452	0.309	-1.463
Male : Expecting	-0.142	0.629	-0.226	-	-	-	-0.336	0.083	-4.061	-0.825	0.309	-2.674
Male : Parent	-2.136	0.629	-3.398	-	-	-	-0.080	0.114	-0.702	-1.563	0.310	-5.040
<i>P. leucopus</i> : Mated	0.125	0.864	0.144	-1.034	0.722	-1.432	0.172	0.117	1.469	-	-	-
<i>P. polionotus</i> : Mated	0.890	0.889	1.001	0.541	0.743	0.729	0.085	0.121	0.700	-	-	-
<i>P. californicus</i> : Mated	1.454	0.920	1.580	1.027	0.769	1.335	0.118	0.114	1.038	-	-	-
<i>P. leucopus</i> : Expecting	0.701	0.864	0.812	-0.521	0.722	-0.721	0.328	0.117	2.805	-	-	-
<i>P. polionotus</i> : Expecting	0.947	0.889	1.065	0.325	0.743	0.437	0.384	0.121	3.170	-	-	-
<i>P. californicus</i> : Expecting	2.316	0.920	2.517	1.713	0.769	2.227	0.109	0.114	0.962	-	-	-
<i>P. leucopus</i> : Parent	-0.830	0.864	-0.961	-1.468	0.722	-2.033	0.172	0.117	1.469	-	-	-
<i>P. polionotus</i> : Parent	-1.573	0.889	-1.770	-1.608	0.743	-2.165	0.502	0.121	4.147	-	-	-
<i>P. californicus</i> : Parent	2.974	0.920	3.233	2.680	0.769	3.484	0.098	0.090	1.086	-	-	-

14 **Table 3 - Post-hoc comparisons of Species : Reproductive Stage interaction levels for time spent huddling.**

15 For the sake of space, only significant differences are displayed ( $p < 0.05$ ).

Comparison	Estimate	SE	z value	p
Parent <i>P. maniculatus</i> - Virgin <i>P. maniculatus</i>	2.692	0.656	4.107	3.614E-03
Parent <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	3.865	0.836	4.623	3.520E-04
Virgin <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	4.192	0.860	4.872	1.070E-04
Mated <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	4.737	0.860	5.507	3.870E-06
Expecting <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	4.574	0.860	5.318	1.090E-05
Parent <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	5.311	0.860	6.174	7.540E-08
Parent <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	6.230	0.890	6.997	3.070E-10
Parent <i>P. maniculatus</i> - Mated <i>P. maniculatus</i>	3.036	0.656	4.631	3.420E-04
Parent <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	4.209	0.836	5.034	4.800E-05
Virgin <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	4.535	0.860	5.272	1.380E-05
Mated <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	5.081	0.860	5.907	3.840E-07
Expecting <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	4.918	0.860	5.717	1.180E-06
Parent <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	5.655	0.860	6.573	5.660E-09
Parent <i>P. californicus</i> - Mated <i>P. maniculatus</i>	6.574	0.890	7.383	1.830E-11
Parent <i>P. maniculatus</i> - Expecting <i>P. maniculatus</i>	3.256	0.656	4.967	6.730E-05
Parent <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	4.429	0.836	5.298	1.210E-05
Virgin <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	4.756	0.860	5.528	3.460E-06
Mated <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	5.302	0.860	6.163	8.000E-08
Expecting <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	5.139	0.860	5.973	2.580E-07
Parent <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	5.875	0.860	6.829	9.890E-10
Parent <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	6.794	0.890	7.630	2.800E-12
Parent <i>P. californicus</i> - Parent <i>P. maniculatus</i>	3.538	0.890	3.973	6.171E-03
Parent <i>P. polionotus</i> - Virgin <i>P. leucopus</i>	3.308	0.836	3.957	6.524E-03
Parent <i>P. californicus</i> - Virgin <i>P. leucopus</i>	4.227	0.867	4.876	1.060E-04
Mated <i>P. polionotus</i> - Mated <i>P. leucopus</i>	2.954	0.836	3.533	3.404E-02
Parent <i>P. polionotus</i> - Mated <i>P. leucopus</i>	3.527	0.836	4.219	2.229E-03
Parent <i>P. californicus</i> - Mated <i>P. leucopus</i>	4.447	0.867	5.129	2.950E-05
Parent <i>P. polionotus</i> - Expecting <i>P. leucopus</i>	3.171	0.836	3.793	1.250E-02
Parent <i>P. californicus</i> - Expecting <i>P. leucopus</i>	4.090	0.867	4.718	2.290E-04
Virgin <i>P. californicus</i> - Parent <i>P. leucopus</i>	-3.301	0.867	-3.808	1.192E-02
Virgin <i>P. californicus</i> - Virgin <i>P. polionotus</i>	-3.628	0.890	-4.074	4.059E-03
Virgin <i>P. californicus</i> - Mated <i>P. polionotus</i>	-4.174	0.890	-4.687	2.630E-04
Mated <i>P. californicus</i> - Mated <i>P. polionotus</i>	-3.064	0.890	-3.441	4.753E-02
Virgin <i>P. californicus</i> - Expecting <i>P. polionotus</i>	-4.011	0.890	-4.504	6.120E-04
Virgin <i>P. californicus</i> - Parent <i>P. polionotus</i>	-4.747	0.890	-5.331	1.020E-05
Mated <i>P. californicus</i> - Parent <i>P. polionotus</i>	-3.637	0.890	-4.085	3.923E-03
Parent <i>P. californicus</i> - Virgin <i>P. californicus</i>	5.667	0.701	8.085	7.990E-14
Parent <i>P. californicus</i> - Mated <i>P. californicus</i>	4.557	0.701	6.501	9.070E-09
Parent <i>P. californicus</i> - Expecting <i>P. californicus</i>	3.915	0.701	5.586	2.510E-06

16 **Table 4 Post-hoc comparisons of Sex : Reproductive Stage interaction levels for time spent huddling.** For  
 17 the sake of space, only significant differences are displayed ( $p < 0.05$ ).

Comparison	Estimate	SE	z value	p
Parent Female - Virgin Female	3.455	0.431	8.013	3.110E-14
Parent Female - Mated Female	3.396	0.431	7.876	8.990E-14
Parent Female - Expecting Female	3.027	0.431	7.021	5.710E-11
Virgin Male - Parent Female	-4.359	0.624	-6.983	7.230E-11
Mated Male - Parent Female	-3.942	0.624	-6.315	6.200E-09
Expecting Male - Parent Female	-4.025	0.624	-6.449	2.700E-09
Parent Male - Parent Female	-2.846	0.624	-4.560	1.120E-04
Parent Male - Virgin Male	1.512	0.431	3.507	9.506E-03

18

19 **Table 5 - Post-hoc comparisons of *Species* : *Reproductive Stage* interaction levels for time spent licking.** For  
20 the sake of space, only significant differences are displayed ( $p < 0.05$ )

Comparison	Estimate	SE	z value	p
Virgin <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	2.266	0.657	3.452	4.508E-02
Virgin <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	3.699	0.676	5.475	4.780E-06
Mated <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	3.762	0.676	5.568	2.830E-06
Expecting <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	3.611	0.676	5.345	9.770E-06
Parent <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	2.896	0.676	4.286	1.728E-03
Parent <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	3.968	0.699	5.674	1.550E-06
Virgin <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	2.744	0.657	4.180	2.710E-03
Virgin <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	4.177	0.676	6.182	7.330E-08
Mated <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	4.240	0.676	6.276	4.140E-08
Expecting <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	4.089	0.676	6.053	1.610E-07
Parent <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	3.374	0.676	4.994	6.280E-05
Parent <i>P. californicus</i> - Mated <i>P. maniculatus</i>	4.446	0.699	6.358	2.460E-08
Virgin <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	2.679	0.657	4.080	4.096E-03
Virgin <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	4.111	0.676	6.085	1.330E-07
Mated <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	4.174	0.676	6.179	7.430E-08
Expecting <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	4.023	0.676	5.955	2.910E-07
Parent <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	3.308	0.676	4.897	1.020E-04
Parent <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	4.380	0.699	6.264	4.440E-08
Virgin <i>P. polionotus</i> - Parent <i>P. maniculatus</i>	2.893	0.676	4.282	1.738E-03
Mated <i>P. polionotus</i> - Parent <i>P. maniculatus</i>	2.956	0.676	4.376	1.173E-03
Expecting <i>P. polionotus</i> - Parent <i>P. maniculatus</i>	2.806	0.676	4.153	3.023E-03
Parent <i>P. californicus</i> - Parent <i>P. maniculatus</i>	3.162	0.699	4.522	6.120E-04
Virgin <i>P. polionotus</i> - Mated <i>P. leucopus</i>	2.944	0.657	4.484	7.260E-04
Mated <i>P. polionotus</i> - Mated <i>P. leucopus</i>	3.007	0.657	4.580	4.690E-04
Expecting <i>P. polionotus</i> - Mated <i>P. leucopus</i>	2.856	0.657	4.350	1.305E-03
Parent <i>P. californicus</i> - Mated <i>P. leucopus</i>	3.213	0.681	4.719	2.470E-04
Virgin <i>P. polionotus</i> - Expecting <i>P. leucopus</i>	2.365	0.657	3.603	2.646E-02
Mated <i>P. polionotus</i> - Expecting <i>P. leucopus</i>	2.429	0.657	3.699	1.861E-02
Expecting <i>P. polionotus</i> - Expecting <i>P. leucopus</i>	2.278	0.657	3.469	4.296E-02
Parent <i>P. californicus</i> - Expecting <i>P. leucopus</i>	2.635	0.681	3.869	9.613E-03
Parent <i>P. californicus</i> - Parent <i>P. leucopus</i>	2.364	0.681	3.472	4.296E-02
Virgin <i>P. californicus</i> - Virgin <i>P. polionotus</i>	-3.216	0.699	-4.598	4.340E-04
Mated <i>P. californicus</i> - Virgin <i>P. polionotus</i>	-2.667	0.699	-3.814	1.190E-02
Virgin <i>P. californicus</i> - Mated <i>P. polionotus</i>	-3.279	0.699	-4.689	2.830E-04
Mated <i>P. californicus</i> - Mated <i>P. polionotus</i>	-2.730	0.699	-3.904	8.505E-03
Virgin <i>P. californicus</i> - Expecting <i>P. polionotus</i>	-3.128	0.699	-4.473	7.550E-04
Mated <i>P. californicus</i> - Expecting <i>P. polionotus</i>	-2.579	0.699	-3.689	1.917E-02
Virgin <i>P. californicus</i> - Parent <i>P. polionotus</i>	-2.413	0.699	-3.450	4.508E-02
Parent <i>P. californicus</i> - Virgin <i>P. californicus</i>	3.485	0.562	6.205	6.390E-08
Parent <i>P. californicus</i> - Mated <i>P. californicus</i>	2.936	0.562	5.228	1.830E-05
Parent <i>P. californicus</i> - Expecting <i>P. californicus</i>	2.185	0.562	3.890	8.928E-03

21 **Table 6 - Post-hoc comparisons of Species : Reproductive Stage interaction levels for proportion retrieved.**

22 For the sake of space, only significant differences are displayed ( $p < 0.05$ )

Comparison	Estimate	SE	z value	p
Parent <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	0.674	0.116	5.791	7.550E-07
Mated <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	0.422	0.120	3.525	3.561E-02
Expecting <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	0.547	0.120	4.569	4.800E-04
Parent <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	0.719	0.120	6.005	2.120E-07
Parent <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	0.759	0.124	6.126	1.020E-07
Parent <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	0.705	0.116	6.060	1.530E-07
Mated <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	0.453	0.120	3.786	1.364E-02
Expecting <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	0.578	0.120	4.830	1.390E-04
Parent <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	0.750	0.120	6.266	4.220E-08
Parent <i>P. californicus</i> - Mated <i>P. maniculatus</i>	0.790	0.124	6.378	2.080E-08
Parent <i>P. maniculatus</i> - Expecting <i>P. maniculatus</i>	0.328	0.087	3.774	1.400E-02
Expecting <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	0.417	0.116	3.582	2.897E-02
Parent <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	0.736	0.116	6.328	2.850E-08
Mated <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	0.484	0.120	4.047	4.879E-03
Expecting <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	0.609	0.120	5.091	3.700E-05
Parent <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	0.781	0.120	6.527	7.840E-09
Parent <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	0.821	0.124	6.630	3.960E-09
Parent <i>P. leucopus</i> - Parent <i>P. maniculatus</i>	0.408	0.116	3.507	3.755E-02
Parent <i>P. polionotus</i> - Parent <i>P. maniculatus</i>	0.453	0.120	3.786	1.364E-02
Parent <i>P. californicus</i> - Parent <i>P. maniculatus</i>	0.493	0.124	3.982	6.363E-03
Parent <i>P. leucopus</i> - Virgin <i>P. leucopus</i>	0.375	0.082	4.574	4.730E-04
Parent <i>P. polionotus</i> - Virgin <i>P. leucopus</i>	0.420	0.116	3.612	2.614E-02
Parent <i>P. californicus</i> - Virgin <i>P. leucopus</i>	0.460	0.121	3.816	1.222E-02
Parent <i>P. leucopus</i> - Mated <i>P. leucopus</i>	0.486	0.082	5.930	3.340E-07
Parent <i>P. polionotus</i> - Mated <i>P. leucopus</i>	0.531	0.116	4.567	4.800E-04
Parent <i>P. californicus</i> - Mated <i>P. leucopus</i>	0.571	0.121	4.737	2.170E-04
Parent <i>P. leucopus</i> - Expecting <i>P. leucopus</i>	0.319	0.082	3.897	8.976E-03
Virgin <i>P. californicus</i> - Parent <i>P. leucopus</i>	-0.683	0.121	-5.658	1.640E-06
Mated <i>P. californicus</i> - Parent <i>P. leucopus</i>	-0.629	0.121	-5.214	1.940E-05
Parent <i>P. polionotus</i> - Virgin <i>P. polionotus</i>	0.438	0.087	5.031	5.010E-05
Parent <i>P. californicus</i> - Virgin <i>P. polionotus</i>	0.478	0.124	3.856	1.051E-02
Virgin <i>P. californicus</i> - Mated <i>P. polionotus</i>	-0.431	0.124	-3.477	4.154E-02
Virgin <i>P. californicus</i> - Expecting <i>P. polionotus</i>	-0.556	0.124	-4.486	6.960E-04
Mated <i>P. californicus</i> - Expecting <i>P. polionotus</i>	-0.502	0.124	-4.054	4.788E-03
Virgin <i>P. californicus</i> - Parent <i>P. polionotus</i>	-0.728	0.124	-5.873	4.650E-07
Mated <i>P. californicus</i> - Parent <i>P. polionotus</i>	-0.674	0.124	-5.441	5.610E-06
Expecting <i>P. californicus</i> - Virgin <i>P. californicus</i>	0.321	0.093	3.458	4.411E-02
Parent <i>P. californicus</i> - Virgin <i>P. californicus</i>	0.768	0.093	8.260	2.660E-14
Parent <i>P. californicus</i> - Mated <i>P. californicus</i>	0.714	0.093	7.684	1.820E-12
Parent <i>P. californicus</i> - Expecting <i>P. californicus</i>	0.446	0.093	4.803	1.580E-04

23

24 **Table 7 - Post-hoc comparisons of Sex : Reproductive Stage interaction levels for proportion retrieved.** For  
 25 the sake of space, only significant differences are displayed ( $p < 0.05$ ).

Comparison	Estimate	SE	z value	p
Parent Female - Virgin Female	0.617	0.062	9.935	< 2e-16
Parent Female - Mated Female	0.602	0.062	9.684	< 2e-16
Parent Female - Expecting Female	0.453	0.062	7.294	7.21E-12
Virgin Male - Parent Female	-0.688	0.092	-7.507	1.57E-12
Mated Male - Parent Female	-0.688	0.092	-7.507	1.57E-12
Expecting Male - Parent Female	-0.578	0.092	-6.313	6.30E-09
Parent Male - Parent Female	-0.406	0.092	-4.436	0.000183
Parent Male - Virgin Male	0.281	0.062	4.527	0.000131

26



27 **Table 8 - Post-hoc comparisons of Sex : Reproductive Stage interaction levels for nest quality score.** For the  
 28 sake of space, only significant differences are displayed ( $p < 0.05$ ).

Comparison	Estimate	SE	z value	p
Expecting Female - Virgin Female	0.866	0.217	3.987	1.470E-03
Parent Female - Virgin Female	1.489	0.217	6.856	1.990E-10
Parent Female - Mated Female	1.199	0.217	5.519	9.210E-07
Mated Male - Expecting Female	-0.931	0.290	-3.210	2.788E-02
Virgin Male - Parent Female	-1.395	0.292	-4.786	4.080E-05
Mated Male - Parent Female	-1.554	0.290	-5.359	2.170E-06
Expecting Male - Parent Female	-1.352	0.290	-4.663	7.160E-05
Parent Male - Parent Female	-1.466	0.292	-5.028	1.240E-05

29

30

31 **Table 9 - Significance of fixed effect factors on attraction/aversion-related behaviours.**  $\chi^2$ ,  $p$  values and the Akaike Inference Criteria (AIC) are given by the *anova*  
32 function.

Fixed effect	Latency of physical contact			Proportion time in pup zone			Preference Index		
	$\chi^2$	$p$	$\Delta AIC$	$\chi^2$	$p$	$\Delta AIC$	$\chi^2$	$p$	$\Delta AIC$
<i>Species : Sex</i>	0.476	0.924	-5.6	2.878	0.411	-3.1	1.694	0.638	-14.9
<i>Species : Reprod. Stage</i>	21.081	0.012*	3.1	12.967	0.164	-5.0	16.564	0.056	-1.4
<i>Sex : Reprod. Stage</i>	13.318	0.004**	7.4	2.545	0.467	-3.5	4.041	0.257	-2.0
<i>Species</i>	-	-	-	1.360	0.715	-4.6	1.786	0.618	-4.2
<i>Sex</i>	-	-	-	0.364	0.546	-1.63	1E-4	0.991	-2.0
<i>Reprod. Stage</i>	-	-	-	14.071	0.003**	8.07	19.591	2.063E-4***	13.6

33

34 **Table 10 - Model summary for attraction/aversion-related behaviours, after removal of non-significant term(s).** Estimates, standard errors (SE) and *z* values are  
35 extracted using the *summary* function. Estimates are given relative to the reference: *P. maniculatus* female virgin.

Fixed effect levels	Latency of physical contact			Proportion time in pup zone			Preference Index		
	Estimate	SE	<i>z</i> value	Estimate	SE	<i>z</i> value	Estimate	SE	<i>z</i> value
(Intercept)	6.010	0.561	10.868	0.551	0.012	46.720	0.206	0.035	5.827
<i>P. leucopus</i>	-3.860	0.690	-5.595	-	-	-	-	-	-
<i>P. polionotus</i>	-3.975	0.710	-5.599	-	-	-	-	-	-
<i>P. californicus</i>	-2.834	0.735	-3.857	-	-	-	-	-	-
Male	-0.013	0.502	-0.025				-	-	-
Mated	0.280	0.705	0.398	-0.040	0.016	-2.520	-0.170	0.049	-3.443
Expecting	-0.843	0.705	-1.197	-0.058	0.016	-3.710	-0.207	0.049	-4.229
Parent	-2.582	0.705	-3.664	-0.032	0.016	-2.000	-0.124	0.049	-2.527
Male : Mated	0.708	0.630	1.124	-	-	-	-	-	-
Male : Expecting	1.254	0.630	1.989	-	-	-	-	-	-
Male : Parent	2.175	0.630	3.451	-	-	-	-	-	-
<i>P. leucopus</i> : Mated	-0.138	0.866	-0.159	-	-	-	-	-	-
<i>P. polionotus</i> : Mated	-0.075	0.892	-0.084	-	-	-	-	-	-
<i>P. californicus</i> : Mated	-1.078	0.923	-1.168	-	-	-	-	-	-
<i>P. leucopus</i> : Expecting	2.215	0.866	2.556	-	-	-	-	-	-
<i>P. polionotus</i> : Expecting	1.242	0.892	1.394	-	-	-	-	-	-
<i>P. californicus</i> : Expecting	-0.779	0.923	-0.844	-	-	-	-	-	-
<i>P. leucopus</i> : Parent	2.075	0.866	2.394	-	-	-	-	-	-
<i>P. polionotus</i> : Parent	1.488	0.892	1.669	-	-	-	-	-	-
<i>P. californicus</i> : Parent	0.612	0.923	0.663	-	-	-	-	-	-

36

37 **Table 11 - Post-hoc comparisons of *Species : Reproductive Stage* interaction levels for latency to physical**  
38 **contact.** For the sake of space, only significant differences are displayed ( $p < 0.05$ )

Comparison	Estimate	SE	z value	p
Virgin <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	-3.860	0.722	-5.349	9.730E-06
Mated <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	-3.364	0.722	-4.661	3.180E-04
Parent <i>P. leucopus</i> - Virgin <i>P. maniculatus</i>	-3.281	0.722	-4.546	5.370E-04
Virgin <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	-3.975	0.743	-5.353	9.620E-06
Mated <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	-3.415	0.743	-4.599	4.240E-04
Expecting <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	-2.949	0.743	-3.971	6.512E-03
Parent <i>P. polionotus</i> - Virgin <i>P. maniculatus</i>	-3.982	0.743	-5.362	9.200E-06
Virgin <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	-2.834	0.769	-3.687	2.040E-02
Mated <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	-3.277	0.769	-4.264	1.891E-03
Expecting <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	-3.830	0.769	-4.982	6.610E-05
Parent <i>P. californicus</i> - Virgin <i>P. maniculatus</i>	-3.717	0.769	-4.836	1.380E-04
Virgin <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	-4.495	0.722	-6.228	5.670E-08
Mated <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	-3.998	0.722	-5.540	3.470E-06
Expecting <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	-2.496	0.722	-3.459	4.768E-02
Parent <i>P. leucopus</i> - Mated <i>P. maniculatus</i>	-3.915	0.722	-5.425	6.550E-06
Virgin <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	-4.610	0.743	-6.207	6.370E-08
Mated <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	-4.050	0.743	-5.454	5.630E-06
Expecting <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	-3.583	0.743	-4.825	1.440E-04
Parent <i>P. polionotus</i> - Mated <i>P. maniculatus</i>	-4.617	0.743	-6.217	6.040E-08
Virgin <i>P. californicus</i> - Mated <i>P. maniculatus</i>	-3.469	0.769	-4.513	6.210E-04
Mated <i>P. californicus</i> - Mated <i>P. maniculatus</i>	-3.912	0.769	-5.089	3.920E-05
Expecting <i>P. californicus</i> - Mated <i>P. maniculatus</i>	-4.464	0.769	-5.807	7.420E-07
Parent <i>P. californicus</i> - Mated <i>P. maniculatus</i>	-4.352	0.769	-5.662	1.740E-06
Virgin <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	-3.644	0.722	-5.049	4.700E-05
Mated <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	-3.148	0.722	-4.361	1.241E-03
Parent <i>P. leucopus</i> - Expecting <i>P. maniculatus</i>	-3.064	0.722	-4.246	2.025E-03
Virgin <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	-3.759	0.743	-5.061	4.450E-05
Mated <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	-3.199	0.743	-4.308	1.566E-03
Expecting <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	-2.733	0.743	-3.680	2.078E-02
Parent <i>P. polionotus</i> - Expecting <i>P. maniculatus</i>	-3.766	0.743	-5.071	4.270E-05
Mated <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	-3.061	0.769	-3.982	6.282E-03
Expecting <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	-3.613	0.769	-4.701	2.650E-04
Parent <i>P. californicus</i> - Expecting <i>P. maniculatus</i>	-3.501	0.769	-4.555	5.190E-04

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40

41 **Table 12 - Post-hoc comparisons of Sex : Reproductive Stage interaction levels for latency to physical**  
 42 **contact.** For the sake of space, only significant differences are displayed ( $p < 0.05$ ).

Comparison	Estimate	SE	z value	p
Parent Female - Virgin Female	-1.493	0.459	-3.251	2.879E-02
Parent Female - Mated Female	-1.480	0.459	-3.222	3.052E-02
Parent Female - Expecting Female	-1.413	0.459	-3.076	4.826E-02
Mated Male - Parent Female	2.176	0.611	3.563	9.917E-03
Expecting Male - Parent Female	2.654	0.611	4.345	3.890E-04
Parent Male - Parent Female	2.163	0.611	3.541	1.038E-02

43

44

45 **Table 13 - Post-hoc comparisons of the levels of significant terms for the proportion of time spent in the**  
 46 **pup zone and the preference index.** For the sake of space, only significant differences are displayed ( $p < 0.05$ ).

Response variable	Comparison	Estimate	SE	<i>z</i> value	<i>p</i>
Proportion time in pup zone	Expecting - Virgin	-0.058	0.016	-3.713	1.230E-03
Preference index	Mated - Virgin	-0.170	0.049	-3.443	2.875E-03
	Expecting - Virgin	-0.207	0.049	-4.229	1.410E-04
	Parent - Virgin	-0.124	0.049	-2.527	4.602E-02

47

48

49 **Table 14 - Significance of explanatory factors on behaviours measured in the test of attraction/aversion to**  
50 **a pup; in parents that were tested at earlier reproductive stages and parents that were only tested only**  
51 **once.** The residual sums of squares (RSS), *F* statistics and *p* values are given by the *anova* function.

Fixed effect	Proportion time in pup zone			Preference Index		
	$\Delta$ RSS	<i>F</i>	<i>p</i>	$\Delta$ RSS	<i>F</i>	<i>p</i>
<i>Species : Sex</i>	0.004	0.208	0.813	0.194	1.426	0.246
<i>Species : Reprod. Stage</i>	0.018	1.034	0.360	0.155	1.132	0.327
<i>Sex : Reprod. Stage</i>	0.016	1.826	0.180	0.223	3.238	0.075
<i>Species</i>	0.024	1.355	0.263	0.228	1.617	0.205
<i>Sex</i>	0.027	3.038	0.085	0.484	6.762	0.011*
<i>Reprod. Stage</i>	0.081	9.073	0.003**	0.496	6.931	0.009**

52

53 **Table 15 -Model summary for behaviours measured in the test of attraction/aversion to a pup, in parents**  
54 **that were tested at earlier reproductive stages and parents that were tested only once.** Estimates, standard  
55 errors (SE) and  $z$  values are given by the function *summary()*.

Explanatory variable levels	Proportion time in pup zone			Preference Index		
	Estimate	SE	$z$ value	Estimate	SE	$z$ value
(Intercept)	0.339	0.014	23.740	0.314	0.049	6.407
Male	-	-	-	-0.145	0.056	-2.600
Parent	-0.060	0.020	-3.012	-0.147	0.056	-2.633

56