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Social life beyond the rural: conspecific interactions of a non-commensal mouse in urban spaces.

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Abstract

Animals living in urban regions often exhibit characteristics that differentiate them from their counterparts in rural areas. In wild animals, certain behavioral traits, change in response to urbanization conditions, including social tolerance. However, in small species such as mice, information on this topic is sparse and contradictory. Therefore, this study aims to evaluate whether urbanization has a bearing on the degree of social tolerance exhibited by a mouse species found in urban areas in the center of Mexico. We studied three urban and three rural populations of the Oaxacan rock deer mouse (*Peromyscus amplus*), a species that has recently been found inhabiting both types of environments. Social tolerance was assessed through the frequency of aggressive behaviors and the duration of affiliative behaviors. These measures were recorded during 10-min paired confrontations in a neutral arena between 84 adult mice (38 urban, 46 rural) captured over one year in both rainy and dry seasons; opponents were randomly paired within the same zone (urban–urban or rural–rural) and, whenever possible, from different sampling points to minimize familiarity and kinship effects. Results showed no significant differences in levels of social tolerance between urban and rural populations of *P. amplus*. Furthermore, social tolerance was influenced by the individuals´ gender and by climatic season. These results indicate that sex and climatic season, rather than the degree of urbanization, are important factors shaping social tolerance in *P. amplus*. Therefore, it is possible that this species does not exhibit an adaptive behavioral response to urbanization.

Introduction

Urbanization is one of the most widespread processes of environmental modification worldwide, and one of the fastest-growing (Grimm et al. 2008). Urbanized environments have created varied challenges for wildlife, such as new biotic interactions (e.g., competitors, predators, and parasites), anthropogenic stressors (e.g., artificial light, urban noise, and chemical pollutants), novel habitats and new food sources (McDonnell and Hahs 2015). Few species can thrive in these highly transformed habitats (Francis and Chadwick 2012), especially in ecosystems where species richness has dramatically declined because of the rapid human-generated environmental change (Grimm et al. 2008). However, a small number of species can cope with the challenges imposed by these environments and even thrive in the face of them (Magle et al. 2012).

Animals living in urban regions often exhibit characteristics that differentiate them from their counterparts in rural areas (Francis and Chadwick 2012). Behavioral changes can be cited as an example of this phenomenon, with such changes being attributable to environmental challenges engendered by urbanization (Miranda 2017). Urban animals are more inclined to approach humans, explore larger areas, remain active for longer periods, take greater risks, rely on anthropogenic food sources, and utilize human-made structures for nesting or shelter (Chavez-Zichinelli et al. 2013; Mikula et al. 2018; Thomas et al. 2018; Gallo et al. 2019; Palacio 2020). However, the behavioral aspects influencing intrapopulation social relationships in these environments (Lacy and Martins 2003; Baxter-Gilbert and Whiting 2019) have received scant attention. Social tolerance is of paramount importance in

group-living animals, as it dictates the capacity of conspecifics to coexist in proximity to valuable resources without exhibiting aggressive behavior (Cronin and Sánchez 2012). There is evidence that urbanization can influence the interaction patterns among conspecifics; however, the extent to which such an effect can result in diminished or augmented levels of social tolerance remains uncertain (Miranda et al. 2013; Łopucki et al. 2021). Two hypotheses could explain the changes in social tolerance in urban animals; the first one posits that residing in an urban setting may foster a diminished level of social tolerance (Lowry et al. 2013; Baxter-Gilbert and Whiting 2019). This behavioral change is primarily attributed to the distribution of food resources and suitable spaces for individuals. In urban ecosystems, human-provided food resources are often abundant and constant (Lapiedra et al. 2017; Łopucki et al. 2021). However, the spaces that individuals can use are generally much more limited than in rural ecosystems (Baxter-Gilbert and Whiting 2019; Marty et al. 2019). These factors collectively contribute to higher population densities, which facilitate greater conspecific interaction compared to what may be observed in a rural area (Baxter-Gilbert and Whiting 2019; Marty et al. 2019). These characteristics result in an increase in aggressive behaviors and a decrease in affiliative behaviors in urban populations (Parker and Nilon 2008; Lowry et al. 2013). The second hypothesis is essentially the converse, proposing that urban living may engender a greater degree of social tolerance (Lapiedra et al. 2017; Łopucki et al. 2021). This is attributed to the fact that the constant availability of food of anthropogenic origin may decrease intraspecific competition in urban environments, thereby shaping interactions where affiliative behaviors predominate (Marty et al. 2019; Łopucki et al. 2021).

Studies conducted on small-sized species have yielded contradictory results as to the direction in which urbanization affects their social tolerance. For example, in the Australian water dragon (*Intellagama lesueurii*), urbanization results in higher population density and more frequent conspecific combat (Baxter-Gilbert and Whiting 2019). Individuals of the Cuban rock iguana (*Cyclura nubila*) inhabiting high anthropogenic usage sites exhibit more aggressive behavior than individuals inhabiting low anthropogenic usage areas (Lacy and Martins 2003). Likewise, gray squirrels from an urban park show a positive association between density and intraspecific aggression (Parker and Nilon 2008). In this case, all these species showed lower tolerance levels in urban areas compared to rural areas. Conversely, brown anole (*Anolis sagrei*), a solitary and territorial species in which males typically defend perch sites year-round, is less aggressive in urban areas than those found in nearby forests (Husak et al. 2007; Dunham and Wilczynski 2014; Lapiedra et al. 2017). Similarly, urban brown anoles were found to be not only less aggressive but also more tolerant of human presence, bolder following simulated predator encounters, and more exploratory of novel environments than their forest counterparts (Lapiedra et al. 2017). Similar results were observed in the striped field mouse (*Apodemus agrarius*), which exhibits greater social tolerance in urban populations than in rural ones; it is suggested that its behavioral adaptation to urban life is determined by an increase in tolerance rather than by a reduction in aggression per se. (Łopucki et al. 2021). However, most of the aforementioned species are considered synurban species, which show a preference for urban environments, although they can be found in rural areas (Santini et al. 2019).

Unlike recognized widely mobile synurbic species such as birds, small flightless species have a low dispersal capacity, making it difficult for them to leave urbanized areas (Lacy and Martins 2003; Parker and Nilon 2008; Miranda 2017). Small size and the reduced vagility of some species could exacerbate the effects of urbanization on their populations (Miranda 2017; Baxter-Gilbert and Whiting 2019). Within small species, mice have been particularly understudied within this paradigm, particularly given that mice are one of the most diverse groups in the world (Wilson and Reeder 2005). The notion that all mice should be considered a synurban species and that they can adapt indiscriminately to anthropogenic disturbances is a pervasive one. Although this notion is erroneous, it has contributed to the paucity of knowledge regarding the potential impact of urbanization on the social tolerance of wild mouse populations (Łopucki et al. 2021). During an exploratory sampling conducted in some urbanized zones in the state of Tlaxcala, Mexico, the Oaxacan rock deer mouse (*Peromyscus amplus*) was found inhabiting various urban sites (Osorio-Cordero et al. 2025, in review). This species was also observed in disparate rural areas within the region (Osorio-Cordero et al. 2025, in review), which would allow a comparison of the behavioral differences between urban and rural populations. Therefore, the objective of this study is to evaluate whether urbanization has a bearing on the degree of social tolerance exhibited by the Oaxacan rock deer mouse *P. amplus*. We test the hypothesis that urbanization favors a decrease in the social tolerance of *P. amplus*. Consequently, we expect urban populations to show a higher prevalence of intraspecific aggressive interactions, while rural populations should show a higher frequency of affiliative behaviors.

Methods

Study areas

The study was conducted in the community of Chiautzingo, Tetla (N: 19. 46120, W: -98. 10635), a municipality of Tlaxcala, situated in the central highlands of Mexico, at an elevation of 2440 meters above sea level. This community covers an area of about 210 hectares, with a population estimated to be 2000 individuals This community covers an area of approximately 210 hectares and has an estimated human population of 2,000 (Municipality of Tetla de la Solidaridad 2025; INEGI 2020). Mice were captured in two zones. Urban sites were green spaces located within the built-up area of the village, among inhabited dwellings, whereas rural sites were patches of native vegetation remnants located more than two kilometers from the urban area. Urban areas were characterized by inhabited houses, continuous human activity, and the presence of artificial light and anthropogenic noise. In the rural area, there were no human settlements, no anthropogenic noise, and no artificial light. The region's climate is classified as temperate sub-humid, and its predominant ecosystem is a juniper forest, comprising *Juniperus deppeana*. Additionally, the area features oak forests, which are characterized by *Quercus laeta*, *Q. obtusata*, and *Q. crassipes* (Municipality of Tetla de la Solidaridad 2025).

Study animals

During the study, 38 adult individuals were captured in the urban zone (20 males and 18 females), and 46 individuals were captured in the rural zone (23 males and 23 females). Of the subjects that were the focus of statistical analysis, 10 females and 10 males were captured in the urban area during the wet season, and 11 females and 13 males were captured during the dry season. Concurrently, in the rural area, 12 females and 12 males were captured during the wet season, and 13 females and 12 males were captured during the dry season. Some individuals were captured during both the wet season and the dry season.

Capture of *Peromyscus amplus* individuals

From April 2022 to March 2023, a capture-mark-recapture approach was employed to sample the populations of mice from urban and rural areas. Three sampling points were established within the urban area and three in the rural area. Each sampling point comprised an area of approximately 0.5 hectares. The sampling points in the urban area were placed within the town in cultivated areas and remnants of original vegetation close to the ravine area that crosses the urban area. The sampling points in the rural zone were settled in cropland and grassland areas. The sampling points within the urban and rural zones were located at least 300 meters apart to ensure that they represented different mouse populations (Fig. 1). At each sampling point, 30 Sherman traps were set up in three rows, with 10 meters between each trap. The traps were baited with oat flakes and left in place for two consecutive nights, once a week. During each trapping session, trapping was carried out in two urban and two rural sampling points, always maintaining the same sampling effort for each point. In both urban and rural areas, traps were set between 17:00 and 19:00 hours and subsequently collected in the morning between 07:00 and 09:00 hours. In the first capture of each mouse, individuals were sexed, their reproductive status was determined, and they were marked with a unique identification number using a pattern of ear notches. Additionally, standard morphometric measurements were recorded, including left ear length, left hind foot length, vertebral tail length, total body length, and body weight (Vázquez et al. 2020). Data obtained were used to identify the species and the identity of the recaptured individuals. In addition, it allowed us to discard juvenile individuals (those exhibiting grayish pigmentation devoid of additional coloration patterns and weighing less than 23 grams) from the behavioral tests. Body measurements also provided a quantitative assessment of body condition, calculated by dividing total weight by total height (Jakob et al. 1996).

Behavioral test

After data collection, captured mice remained inside the traps in which they were caught until the behavioral tests were conducted at dusk, which avoided additional handling and transfer to new enclosures before testing. The traps were placed in a warm room to prevent thermal stress, since small mammals confined in metal traps are susceptible to heat loss during the cold nights, particularly in the dry season. Individuals were provided with food ad libitum (oat flakes and lettuce as a moisture source, following Sikes et al. 2016), so that hunger and dehydration would not influence their motivation to engage in social interactions and the energetic state of the animals would be comparable among

individuals. Behavioral tests were conducted at night, since *Peromyscus* are strongly nocturnal (Layne 1971) and this period coincides with their natural activity phase. Behavioral tests were conducted between 20:00 and 02:00 hours, after which the individuals were returned to their traps. Although confinement in live traps is known to elevate glucocorticoid levels in *Peromyscus* after more than 4 h (Harper and Austad, 2001), holding time and conditions were identical for all individuals regardless of sex, zone, or sampling point. Because this potential source of stress did not vary systematically across the compared groups, it cannot account for the specific behavioral differences reported here. Subsequently, the individuals were released at dawn in the place where they had been captured. In order to assess the level of social tolerance, paired confrontations were carried out using only adult individuals. Confrontations were conducted between individuals from different sampling points to reduce the effect of familiarity or kinship between opponents. However, when it was impossible to confront individuals from different sampling points, individuals captured from the same population at a distance greater than 100 meters from each other were confronted (a distance that exceeds the average home-range diameter reported for congeneric Peromyscus, 100 ± 25 m; Abramson et al. 2006). Confrontations were carried out between individuals from the same zone (urban vs. urban zone and rural vs. rural zone). The combinations used for each dyad were female vs. female, male vs. male, and female vs. male. Since the confrontations were both intra- and intersexual, both the choice of individuals to confront and the assignment of the dyadic combination, were performed randomly.

The confrontations took place weekly for two consecutive nights and were videotaped for later analysis. They took place in a circular neutral arena made of an acrylic sheet with a radius of 50 cm and a height of 70 cm. The arena had a removable partition and a sawdust bed. The sawdust bed was changed at the end of each confrontation to keep odor contamination to a minimum. Individuals remained inside the arena, separated by an opaque acrylic sheet, for 10 minutes to familiarize themselves with the environment. After this time, the dividing plate was removed, and interactions between individuals were videotaped for 10 minutes (Vázquez et al. 2020) with a closed-circuit camera, using night mode and in complete darkness to avoid interfering with the behavior of the individuals. The camera was installed at a height of one meter above the ground, where the camera's view could cover the entire area of the arena. Only adult individuals were selected for behavioral confrontations. During the testing phase, subjects were involved in various confrontations, both as the focal individual and as opponents. Data from all individuals were used in the subsequent statistical analyses.

Once the videos were obtained, 30% of the videos from the urban area and 30% from the rural area were used to develop the ethogram corresponding to the categories of aggressive behavior (attacking or threatening behaviors that may or may not include physical contact) and affiliative behavior (friendly or sociable behaviors that favor social cohesion). Thus, four aggressive behaviors and three affiliative behaviors were recorded (Table 1). Subsequently, using Solomon Coder video analysis software (Péter 2017; beta 17.02.15), the frequency and duration of aggressive and affiliative behaviors were recorded for all videos obtained. The behavior of each mouse was observed and recorded through a focal analysis. Aggressive behavior was considered as the frequency of attack positions, attacks, chases, and fights displayed by the individuals, while affiliative behavior was considered as the total time that the

individual dedicated to social investigation, huddling, and self-grooming during the test. Finally, once we had the frequency and duration of each behavior, they were summed up and grouped within their behavioral category, leaving only two behavioral variables (aggression and affiliation).

Table 1
Description of aggressive and affiliative behaviors in *P. amplus* individuals.

Category	Behaviors	Description
Aggressive	Attack position	At the opponent's proximity, the individual stands up on its hind legs, placing the front legs in a boxing position.
	Attack	Kicks, bites, pushes, or thrusts made towards the opponent.
	Chase	The individual persistently follows his retreating opponent for at least 3 seconds.
	Fight	A set of continuous attacks against the opponent.
Affiliative	Social investigation	The individual repeatedly sniffs his opponent within approximately 5 cm.
	Huddling	The individual approaches and maintains body contact with his opponent without displaying aggressive behavior.
	Self-grooming	In no particular order, the individual grooms and scratches himself with the muzzle and front and hind legs.

Statistical analysis

As a first step, all response variables (behaviors listed in Table 1) were categorized into ordinal scores to reduce the skewness of their distributions and the influence of extreme values. Ordinal variables were constructed using both the frequency and duration data for the behaviors; these were created by grouping the data in a way that accounted for the skewness of the original distributions, with the aim of reducing it. Frequency data were assigned to eight classes (0–1 = 1, 2–3 = 2, 4–5 = 3, 6–7 = 4, 8–9 = 5, 10–15 = 6, 16–20 = 7, 21–30 = 8), and duration data (s) to eleven classes (0 = 0, 0.1–1 = 1, 1.1–10 = 2, 10.1–30 = 3, 30.1–60 = 4, 60.1–100 = 5, 100.1–150 = 6, 150.1–210 = 7, 210.1–280 = 8, 280.1–360 = 9, > 360.1 = 10). Subsequently, we reduced the number of dependent variables using principal component analysis (PCA). The suitability of the variables for PCA was verified using Bartlett's sphericity test, the Kaiser-Meyer-Olkin (KMO) criterion of sampling adequacy, and the anti-image matrix (Field, 2012).

The PCA yielded three main components that explained 59.72% of the variance (Table 2). The first component explained most of the variance associated with social investigation and huddling behaviors (Table 3). The second component was associated with attack stance and attack behaviors (Table 3), and the third component was associated with self-grooming and environmental exploration (Table 3). We will refer to these components as PC1 (affiliative behavior), PC2 (aggressive behavior), and PC3 (self-grooming and exploration).

Table 2
Total variance explained by the principal components.

Initial self-values			
Component	**Total**	**% variance**	**% cumulative**
1	1.90	23.85	23.85
2	1.51	18.90	42.75
3	1.35	16.96	59.72

Table 3
Rotated component matrix. Obtained using the Varimax rotation method with Kaiser normalization.

	Component		
	1	**2**	**3**
Attack position	0.02	**0.83**	-0.02
Attack	-0.17	**0.84**	-0.11
Chase	-0.41	0.10	0.23
Fight	-0.10	0.15	-0.63
Social investigation	**0.83**	0.17	0.27
Huddling	**0.86**	-0.19	-0.00
Self-grooming	-0.20	-0.16	**0.70**
Environmental exploration	0.05	0.12	**0.67**

Components 1 and 3 showed a normal distribution, for which they were analyzed using mixed linear models. However, component 2 showed several negative values that deviated from the mean, so we opted to use a robust model that gives more weight to values within the normal distribution than to outliers (as described below). For all models, the variables area (rural and urban), sex of the focal individual and of the opponent, body mass index (g/cm^2), and season (time of the year when the subject was captured and tested- rainy or dry) were considered as fixed factors. Body condition was included as a fixed factor (covariate) since an individual's energy state can influence its motivation to engage in agonistic interactions and its ability to compete for resources, as has been observed in the European field vole (*Myodes glareolus*; Erixon et al. 2024). Our observations of the species at this location suggest that individuals tend to be more aggressive during the rainy season (the breeding season) than during the dry season (unpublished data), therefore, season (rainy, dry) was considered as a factor. Since some individuals were tested more than once, individual identity was considered a random effect. All analyses started from the general model with all variables, and backward elimination was performed based on the

“P” and “t” values. To do this, the least significant variables within the model were discarded one by one until the minimum model containing only those variables that explained a significant proportion of the variation in the data was reached. However, for the s *PCA2 (aggressive behavior)*, only the “*t*” values were taken into account to eliminate variables until the final model was reached. This is because, due to the type of probability estimates, the robust model does not use “*P*” or “F” values. The models were run with the R lme4 and robust lmm packages for mixed linear models (lmer) and robust mixed linear models (rlmer), respectively (Bates et al. 2015; Koller 2016). For interactions reaching significance or marginal significance, post-hoc pairwise comparisons among factor-level combinations were performed on estimated marginal means with Tukey adjustment for multiple comparisons, using the R package emmeans (Lenth 2024).

Results

None of the PCAs were significantly related to urban or rural capture areas (all P > 0.06; Table 4). However, only for self-grooming and environmental exploration, a marginally significant interaction between the season and area was identified (P = 0.06; Table 4), suggesting that both behaviors are more prevalent during the dry season in the urban area (β = 0.37 ± 102.75; Table 4). However, Tukey-adjusted post-hoc comparisons among the four season-area combinations revealed no significant pairwise difference (all P > 0.48; Table S1). On the other hand, there were differences associated with sex, with affiliative and aggressive behaviors being lower in males (affiliative behavior, β = -0.48 ± 0.16, Fig. 2a; aggressive behavior, β = -0.63 ± 0.24, Fig. 2b; Table 4). The model also indicated an interaction effect between sex and season (Table 4). That is, males engaged in more affiliative behaviors than females during the dry season, but females showed more affiliative and aggressive behaviors than males during the wet season (affiliative behavior, β = 1.32 ± 0.32; aggressive behavior, β = 0.53 ± 0.22; Table 4; Fig. 3).

Table 4
Results of linear mixed models. The table shows the results of all the fixed effects analyzed in the model.

Intercept	***PCA1 (affiliative behavior)***			***PCA2 (aggresive behavior)***			***PCA3 (self-grooming and exploration)***		
	Estimate	***t value***	***P***	***Estimate***	***t value***	***P***	***Estimate***	***t value***	***P***
	0.17 ± 0.69	**0.24**	**0.80**	**0.80 ± 0.77**	**1.03**	**0.30**	**0.09 ± 0.79**	**0.11**	**0.90**
Zone	0.37 ± 0.24	1.49	0.13	0.02 ± 0.16	0.17	-	-0.33 ± 0.28	-1.19	0.23
Sex (Male)	**-0.63 ± 0.24**	**-2.56**	**0.01**	**-0.48 ± 0.16**	**-2.86**	-	-0.39 ± 0.26	-1.50	0.13
Body mass	-1.37 ± 1.45	-0.94	0.34	-0.68 ± 1.03	-0.65	-	0.79 ± 1.66	0.47	0.63
Season	0.12 ± 0.23	0.53	0.59	-0.22 ± 0.15	-1.42	-	-0.39 ± 0.26	-1.46	0.14
Season (D): **Sex (M)**	**1.32 ± 0.32**	**4.07**	**< 0.001**	**0.53 ± 0.22**	**2.41**	-	0.13 ± 0.37	0.36	0.71
Season (D): Zone (U)	-0.48 ± 0.33	-1.44	0.15	0.03 ± 0.24	0.16	-	0.68 ± 0.37	1.84	0.06
Random effect (ID)	**Variance**			**Variance**			**Variance**		
	0.14			0.07			0.08		

*P values are not reported for the aggressive behavior (PC2) model because robust mixed-effects models fitted via robustlmm (*Koller 2016*) lack an established finite-sample reference distribution for significance testing; only point estimates, standard errors, and t values are given. As a conventional approximation, $|t| \gtrsim 2$ corresponds to $P < 0.05$ under a standard normal (Wald) approximation.*

Post-hoc comparisons (Table S1) showed that sex differences depended on the season. In the wet season, females scored higher than males for both affiliative (P = 0.013) and aggressive behavior (P = 0.004); in the dry season, the sexes no longer differed in aggressive behavior (P = 0.722), and the pattern reversed for affiliative behavior, with males scoring higher than females (P = 0.003). This seasonal shift was evident in males, which increased markedly in affiliative behavior from the wet to the dry season (P < 0.001) and showed a slight increase in aggressive behavior (P = 0.045), whereas females did not change across seasons in either behavior (P = 0.602 and P = 0.154, respectively).

The sex of the opponent and body mass index did not influence any of the behaviors analyzed in the individuals (Table 4). Similarly, the variation in self-grooming and exploration was not explained by the climatic season (Table 4).

Discussion

The findings of this study indicated that there were no substantial disparities in the degree of social tolerance exhibited by urban and rural mice. The results do not support the previous findings in other small species, such as the Australian water dragon (*Intellagama lesueurii*), the Cuban land iguana (*Cyclura nubila*), and the gray squirrel (*Sciurus carolinensis*), in which more aggressive behavior was observed in toward their conspecifics than individuals from rural areas (Lacy and Martins 2003; Parker and Nilon 2008; Baxter-Gilbert and Whiting 2019). Differences in aggression between rural and urban birds have been widely observed in different studies, which have shown that urban populations are more aggressive than rural ones (e.g. Miranda 2017). However, as stated in the section Introduction, most of these species are synurbic. In mammals, the differentiation in social behavior among populations is often considered part of the acclimatization process to urban environments in synurbic species (Ritzel and Gallo 2020). Thus, our results suggest that *P. amplus* does not exhibit a behavioral adaptive response to urban environments, at least in aggressive behavior.

In species where urban populations are more aggressive than rural ones, the difference in social tolerance has been related to a higher population density in urban areas compared to rural areas (Lacy and Martins 2003; Parker and Nilon 2008; Baxter-Gilbert and Whiting 2019). This is a phenomenon that has been explained by competition for suitable spaces within urban areas, which tend to be finite and much more limited than those in rural areas (Baxter-Gilbert and Whiting 2019). In this study, the population density was not estimated, but the number of adult individuals captured in rural and urban zones was similar, suggesting that density could be similar in both zones. If aggressive behavior responds to the high density of this mouse population, the absence of density differences probably explains the absence of differences in tolerance between rural and urban mouse populations.

Anthropogenic food is another factor that has been considered to explain the low aggressiveness in individuals inhabiting urban zones, which they can take advantage of. If such food is available year-round, it could deter aggressive interactions, as observed in *A. agrarius* mice and *A. sagrei* lizards (Lapiedra et al. 2017; Marty et al. 2019; Łopucki et al. 2021). These species have been observed to be more tolerant of their conspecifics in urban areas than in rural areas due to the constant availability of anthropogenic food, which drastically reduces the level of intraspecific competition (Lapiedra et al. 2017; Marty et al. 2019; Łopucki et al. 2021). However, unlike *A. agrarius* and *A. sagrei*, the urban individuals of our study species did not exhibit greater tolerance than rural individuals, suggesting that the availability of food may not be different between rural and urban zones.

The results also revealed that location does not influence the behavior of the mouse populations in either area, or that they behave as if they were a single population. For both populations, a sex effect was

therefore observed in the mice's behavior, with males displaying lower levels of both aggressive and affiliative behavior than females. This result contrasts with findings in the Southern Mountain cavy (*Microcavia australis*), where males from different populations engage in direct aggressive interactions more frequently than females (Andino et al. 2011). Direct agonistic interactions always involve high energy, time, and health costs (Parker and Nilon 2008; Hurtado and Mabry 2017). It is likely that males in *P. amplus* populations engage in less aggressive behavior as a strategy to reduce their costs.

However, this sex difference was not constant throughout the year. Post-hoc comparisons (Table S1) showed that sex differences depended on the season. In the wet season, females scored higher than males for both affiliative and aggressive behavior; in the dry season, the sexes no longer differed in aggressive behavior, and the pattern reversed for affiliative behavior, with males scoring higher than females. This seasonal shift was evident in males, which increased markedly in affiliative behavior from the wet to the dry season and showed a slight increase in aggressive behavior, whereas females did not change across seasons in either behavior. For this same species (*P. amplus*) from a temperate forest, near the present study area, it is known that individuals tend to cluster more during the dry season than at other times of the year (De-la-Cruz et al. 2019), suggesting more tolerance among individuals. The present study did not assess the spatial proximity between individuals, but they were captured during the driest months (December, January, and February), which also coincided with the coldest months of the year and possibly when individuals could have more tolerance towards the proximity of conespecifics. In addition, it is known that social behavior enables individuals to maintain their body temperature while reducing energy expenditure (Gilbert et al. 2010), as occurs in domestic mice, which increase their huddling time as the ambient temperature drops (Groó et al. 2018). Spatial proximity could favor lower levels of competition during the dry season (Jasso del Toro and Nekaris 2019). Similar results have been observed in mice of the species *Ochrotomys nuttalli* and *Apodemus sylvaticus*, which gather during the winter to withstand adverse environmental conditions, thereby increasing their chances of survival (Springer et al. 1981; Wolton 1985). Then, it is probable that the affiliative behavior (such as huddling) exhibited by the males encourages individuals to stay close to each other in response to the effect of low temperatures of the season. Likewise, an increase in individuals´ aggressive capacity —without ruling out their ability to exhibit affiliative behavior— could enhance their ability to defend some resources, such as their food, which would provide an advantage to *P. amplus* individuals during this season.

The marked seasonal plasticity of the males' behavior contrasted with the stability of females' parallels patterns described in other rodents. In several *Peromyscus* species, male aggression increases under short-day, winter-like photoperiods through estrogen-dependent, non-gonadal pathways, even as testosterone declines with gonadal regression (Trainor et al. 2007; Trainor et al. 2008), offering a plausible physiological route through which season could reshape male social behavior largely independently of breeding condition. A comparable photoperiod effect has been reported in female California mice (*Peromyscus californicus*), although through partly distinct neural mechanisms (Silva et al. 2010). It is likely that the apparent stability of *P. amplus* females across seasons should not be assumed to reflect a general insensitivity of female behavior to season but could instead depend on unmeasured factors such as individual reproductive or estrous state. Furthermore, coupled increases in

affiliative and agonistic behavior, rather than a strict trade-off between them, have also been documented in meadow voles exposed to short days (Lee et al. 2019), consistent with the pattern observed here in which affiliative and aggressive scores rose together in males during the dry season. As no hormonal or photoperiod measurements were taken in this study, these mechanisms remain speculative, and future work directly assessing reproductive condition or photoperiod exposure would help test whether they underlie the sex-specific seasonal plasticity documented here.

In another way, although no differences in the behavior were found between populations, a marginally significant interaction between season and area was identified for the third principal component. Results suggest a trend toward greater self-grooming and exploration during the dry season within the urban area. This increased exploratory behavior has been associated with urbanization in urban common voles (*Microtus arvalis*; Mazza et al. 2020). In the striped field mouse (*Apodemus agrarius*), individuals of urban areas exhibit higher exploration activity than rural counterparts (Dammhahn et al. 2020). However, the Tukey-adjusted post-hoc comparisons did not identify any specific season-area combination that differed significantly from the others (Table S1), so this pattern should be interpreted with caution. As to the adaptive significance of self-grooming behavior found in this study, it is unclear in the context of urbanization's effect on behavioral adaptation. Further studies should aim to confirm whether the behaviors may play a role in the adaptation of individuals of this species to urban conditions.

Likewise, given that the characteristics of the sites where individuals of this species were found are like those of rural areas, in ravines and remnants of natural vegetation, (Pers. Obs.) it might be worth assessing whether the species is undergoing extirpation. In this regard, the results would suggest that the behaviors assessed are not very flexible within the *P. amplus* populations. Moreover, they could demonstrate how species are extirpated from their habitat during urbanization. The results of the present study showed that social tolerance in *P. amplus* is shaped by factors such as climatic season and the sex of individuals, rather than by the degree of urbanization. However, the question arises as to whether the *P. amplus* population might be disappearing from the urban area, which would suggest that it is a remnant population of this species.

Declarations

Competing interests:

The authors declare they have no financial interests. Compliance with ethical standards

Conflict of interest.

The authors declare that they have no conflict of interest.

Ethics declaration

The Dirección General de Vida Silvestre, Secretaría de Medio Ambiente y Recursos Naturales, gave the authorizations (SGPA/DGVS/07931/21) to carry out the fieldwork.

Author contribution

LOC, AB, LRM, JV conceived the idea for this paper. AB, JAF, ARC, LRM, JV reviewed the article critically for important intellectual content. LOC, LRM, JV helped to collect the data. LOC, JAF, ARC help with analysis and interpretation of data. All authors contributed critically to the drafts and gave final approval of the manuscript.

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Data availability

Data will be made available on request.

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Figures

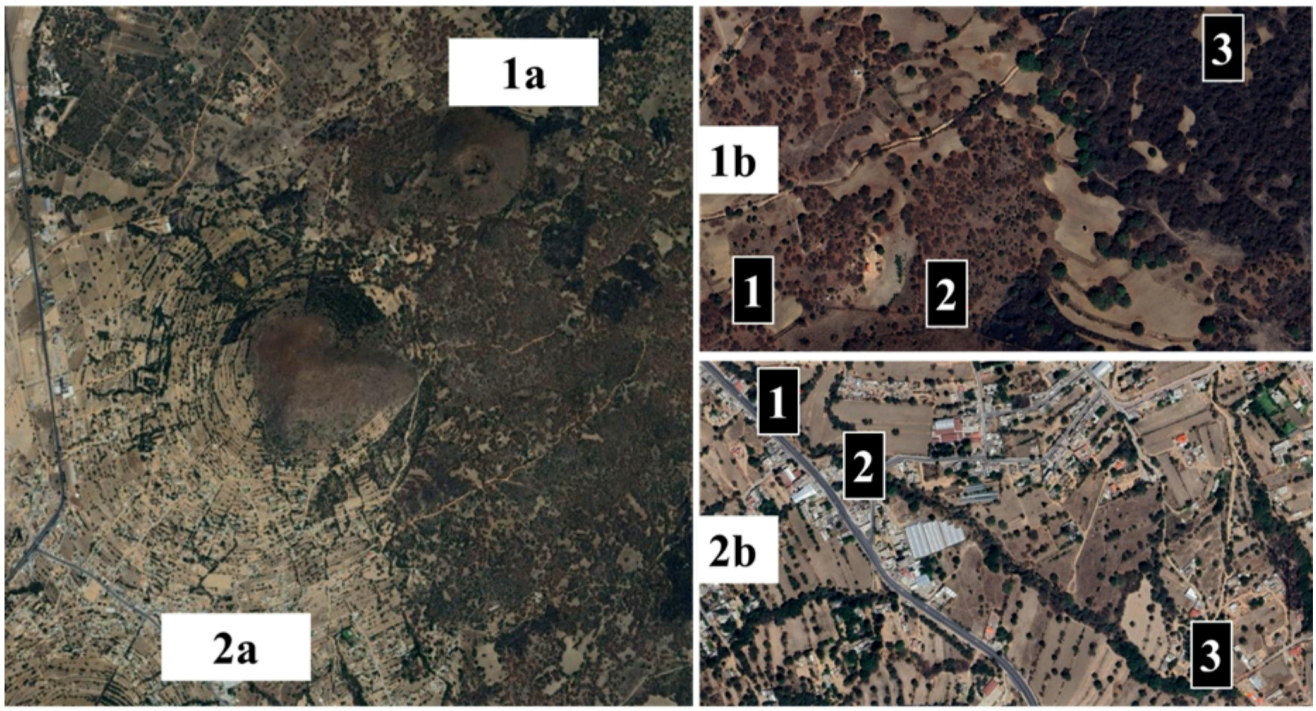


Figure 1

Location of the sampling points. Left: aerial view of the study area showing the rural (rectangle 1a) and urban (rectangle 2a) zones. Right: enlargements of rectangles 1a (1b, rural) and 2a (2b, urban); numbers 1–3 indicate the three sampling points established in each zone.

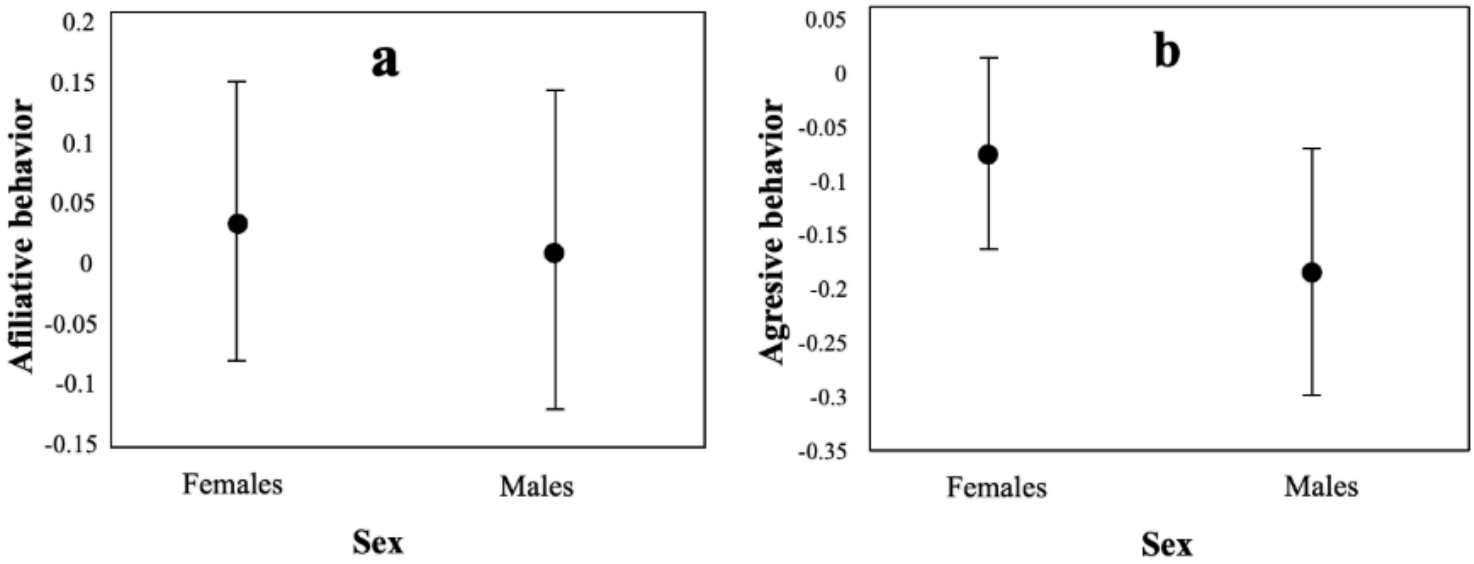


Figure 2

Affiliative (a) and aggressive (b) behavior in females (n = 41) and males (43) of *P. difficilis*. Each figure shows the mean ± standard error and includes both urban and rural individuals.

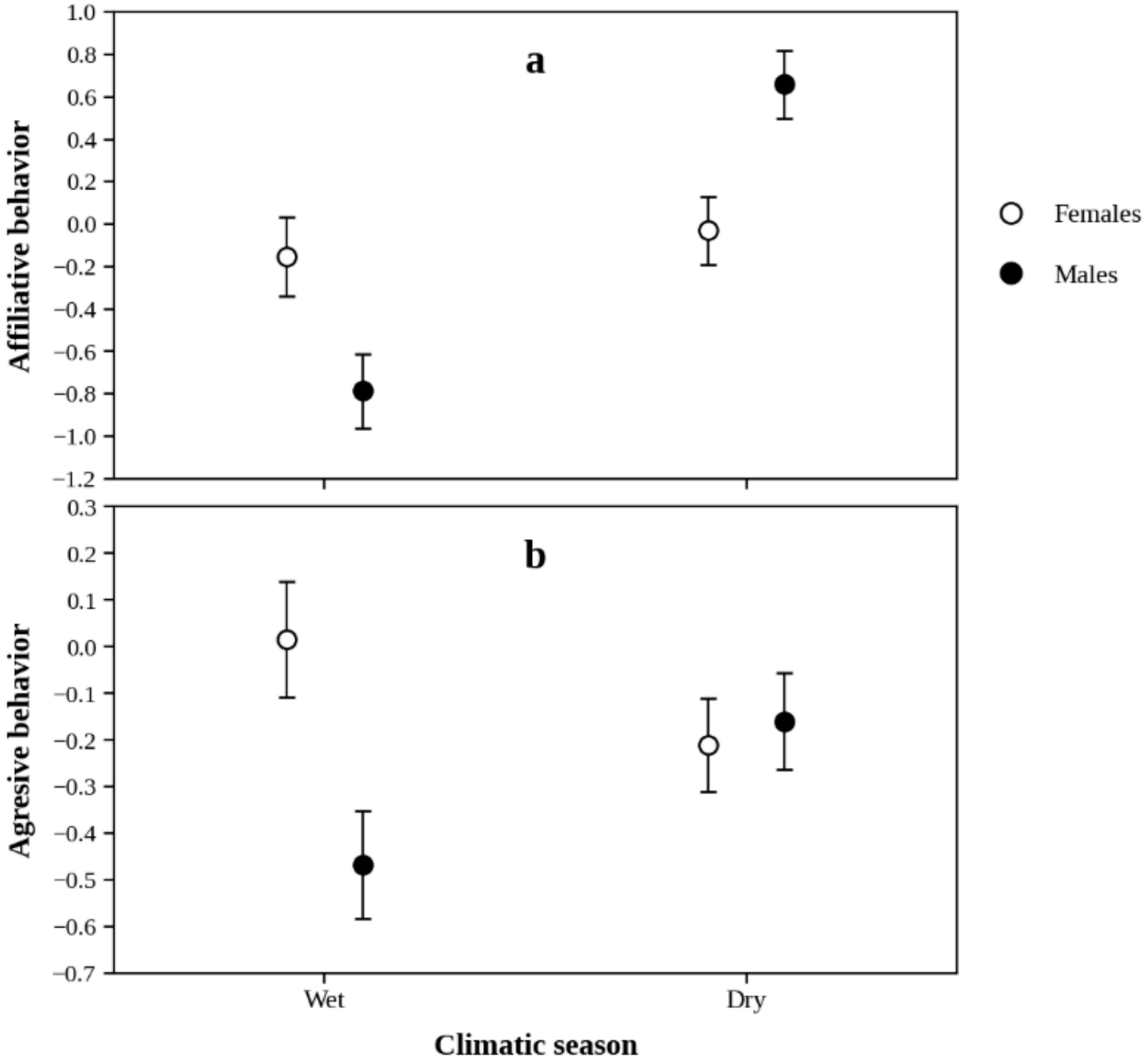


Figure 3

Affiliative (a) and aggressive (b) behavior of *P. amplus* individuals during the wet season and the dry season. Females during wet (n = 22) and dry (n = 24) season. Males during wet (n = 12) and dry (n = 25) season. Each figure shows estimated marginal means ± standard error.

Supplementary Files

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