



## Multiple paternity in a wild population of the corn mouse: its potential adaptive significance for females

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Mating with multiple males within a single reproductive event is a common female mating strategy in mice and voles, but its adaptive function is often unclear. In this study, we used 7 microsatellite loci to investigate if multiple paternity occurs in wild populations of the socially promiscuous *Calomys musculinus*. We also analyzed if multiple paternity increases litter size and/or genetic variability among offspring. In addition, we examined if multiple paternity occurs more frequently in litters conceived at high population density than those conceived at low population density. By genotyping 23 females and their 135 embryos ( $5.9 \pm 1.6$  SE pups per female), we found that 56.5% of the litters were sired by 2 or 3 males. We found no association between multiple paternity, litter size, and genetic variability. In addition, multiple paternity did not vary in relation to population density. Our results provide clear evidence of multiple paternity and offer the first genetic documentation of mating systems in mice in Argentina. We discuss the extent of multiple paternity in relation to potential adaptive strategies in female corn mouse.

El apareamiento con múltiples machos durante un solo evento reproductivo es una estrategia común de acoplamiento en hembras de ratones y ratas de campo, sin embargo su función adaptativa a menudo es poco clara. En este estudio, hemos utilizado 7 loci de microsatélites para investigar si existe paternidad múltiple en poblaciones silvestres del ratón maicero (*Calomys musculinus*) especie socialmente promiscua. También analizamos si la paternidad múltiple aumenta el tamaño de la camada y/o su variabilidad genética. Además, se analizó si la paternidad múltiple ocurre con más frecuencia en camadas concebidas a alta densidad poblacional, que las concebidas a baja densidad poblacional. El genotipado de 23 hembras y sus 135 embriones ( $5.9 \pm 1.6$  ES crías por hembra) demostró que, al menos el 56.5% de las camadas son engendradas por dos o tres machos. No se encontró asociación entre la paternidad múltiple, tamaño de la camada y la variabilidad genética. Además, la paternidad múltiple no varió en relación con la densidad de la población. Nuestros resultados proveen clara evidencia de paternidad múltiple y ofrecen la primera documentación genética de sistemas de apareamiento en ratones en Argentina. Discutimos el alcance de la paternidad múltiple con relación a potenciales estrategias adaptativas en hembras del ratón maicero.

Key words: *Calomys musculinus*, microsatellite, multiple male mating, paternity share

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Promiscuity can be defined as a mating system in which both males and females mate nonexclusively with multiple partners during a breeding season, without precluding the chance of female choice (McEachern et al. 2009). Multiple paternity (or the insemination of a female by more than 1 male during a single reproductive event) is a common result of promiscuity.

Its extent in a population will be affected by 2 main behavioral categories: the ability of males to gain access to already mated females and the degree to which females seek to copulate with several males (Wolff and Macdonald 2004; Parker and Birkhead 2013). In the latter category, females play an active role and their benefits are the driving force behind multiple

paternity (Berteaux et al. 1999). Behavioral and genetic evidence suggests that promiscuous mating systems (or multiple mating) and multiple paternity are common in voles and mice (Baker et al. 1999; Bartmann and Gerlach 2001; Wolff and Dunlap 2002; Wolff et al. 2002; Klemme et al. 2006, 2007; Bryja et al. 2008; Borkowska et al. 2009). However, unlike males, females are not expected to increase their reproductive success by mating with multiple partners (Trivers 1972). Moreover, mating with several males can incur a number of costs for females, in terms of time and energy expenditure, increased predation risks, injuries, and sexually transmitted diseases (Daly 1978; Magnhagen 1991; Rowe 1994; Siva-Jothy 2006). Understanding the selective advantage of promiscuous female mating behavior is an important issue in evolutionary biology because, in a cost–benefit context, it is still difficult to explain why females should readily solicit copulations with multiple males (Stockley 2003). A number of benefits that may outweigh potential costs have been proposed to explain multiple mating of females. These benefits may enhance female fitness either directly (through immediate benefits) or indirectly (through genetic benefits for their offspring). Wolff and Macdonald (2004), Hoogland (2013), and Parker and Birkhead (2013) summarized several nonmutually exclusive hypotheses that explain how females can benefit from multiple mating behavior. Within these hypotheses, one proposes that multimale mating increases litter size (direct benefit), and the other proposes that multimale mating increases genetic variability among offspring (indirect benefit).

Hoogland (1998) and Bryja et al. (2008) found that mating with more than 2 partners increases the probability of conception, resulting in increased litter size. The mechanism by which multimale mating increases conception rates or litter size is still not well understood but may be either to guard against male sterility or sperm depletion (Haig and Bergstrom 1995) or just to stimulate ovulation (Jennions and Petrie 2000; Kraaijeveld-Smit et al. 2002). The importance of inducing ovulation by multiple copula has been examined in some rodent species. A larger number of intromissions resulted in a greater percentage of eggs ovulated in voles (Gray et al. 1977; Milligan 1982) and Norway rats (Zarrow and Clark 1968). However, in many species, multimale mating had no effect on conception rates, and litter size did not tend to be different at birth (Hoogland 1995; Wolff and Dunlap 2002). The latter could be explained through the uncertain paternity hypothesis that assumes that copulation inhibits males from killing the future young pups for a period of time long enough for the young to be weaned (Agrell et al. 1998). Thus, infanticidal males should not kill the offspring of previous sexual partners (Ebensperger 1998; Thonhauser et al. 2013).

The increase of genetic variability per litter in unpredictable environments that results from multiple paternity may also provide a plausible explanation for why females mate with several males (Hanken and Sherman 1981; Watson 1991; Madsen et al. 1992; Murie 1996; Sillero-Zubiri et al. 1996). In a study of the small marsupial species *Antechinus agilis*, Kraaijeveld-Smit et al. (2002) proposed both that offspring sired by multiple

males are more diverse for immune suppression levels and that variation of environmental conditions would favor certain genotypes over others; multimale mating may be an advantage for females. Several studies have shown that multimale matings allow females to increase the genetic variability of their progeny in a wide range of species (Martinez et al. 2000; Calsbeek et al. 2007; King et al. 2014; Thonhauser et al. 2014).

Although multiple paternity typically carries a strong phylogenetic signal (Wusterbarth et al. 2010), multiple paternity rates often vary between populations within 1 species as a consequence of demographic and environmental conditions (Hoogland 1995; Westneat and Stewart 2003; Dean et al. 2006). Due to the fact that in vole species the occurrence of multiple paternity is affected by annual variations on population density and resource availability (Bryja et al. 2008; Ishibashi and Saitoh 2008; McEachern et al. 2009; Eccard et al. 2011), multiple paternity may vary within a population during a breeding season.

In corn mice, *Calomys musculinus* (Cricetidae, Sigmodontinae), a promiscuous mating system has been proposed, based until now on behavioral studies (Laconi et al. 2000; Steinmann et al. 2006a, 2006b, 2009; Sommaro et al. 2010a, 2010b; Coda et al. 2011; Steinmann and Priotto 2011). During the breeding period, *C. musculinus* males have large home ranges that overlap with several females and males, while females keep smaller home ranges crossed by both transient and resident males but never by breeding females (Steinmann et al. 2005, 2006a; Steinmann 2006). Females are the territorial sex, whereas males show high levels of intrasexual tolerance and amicable behaviors (Steinmann et al. 2009). In this species, males do not contribute to nest building, there is no nest cohabitation by a male–female pair, young are highly dependent on parental protection, and parental care is carried out exclusively by females (Cutrera et al. 1988; Yunes et al. 1991). Females show a high frequency of postpartum estrus, which implies that a new pregnancy may overlap with the lactation of the previously produced litter (Sommaro et al. 2009).

Based on available evidence of promiscuity in *C. musculinus* in this study, we test the hypothesis that in wild populations, multiple paternity occurs within litters (genetic promiscuity). In addition, we analyzed if multiple paternity increases litter size and genetic variability within *C. musculinus* broods. Finally, in order to determine whether multiple paternity varies in relation to population density, we also examined if multiple paternity occurs more in litters conceived in the wild in the late breeding season (high population density) than those conceived in the early breeding season (low population density). To our knowledge, this research provides the first genetic documentation of mating systems in mice in Argentina.

## MATERIALS AND METHODS

*Study species.*—*Calomys musculinus* is a short-lived grassland mouse inhabiting Pampean agrarian ecosystems of central and northwestern Argentina that has been studied mainly in relation to its role as reservoir of the Junin virus, the etiological

agent of the Argentine Hemorrhagic Fever. This species is a good settler of disturbed habitats and shows a wider habitat and trophic niche than other coexisting rodent species (de Villafañe and Bonaventura 1987; Sommaro et al. 2010a). Although this rodent can live in a wide variety of habitats including natural pastures, crop, and stubble fields, its primary habitats are the weedy borders along roads, railway banks, and wire fences between cultivated fields or pastures (Busch et al. 2000; Simone et al. 2010; Sommaro et al. 2010a). Corn mouse populations are characterized by seasonal density changes with low density in spring (September–December; 16 individuals/ha) and peaks in late summer (February–March) or mid-autumn (April–May; 260 individuals/ha—Mills et al. 1991; Mills and Childs 1998). Populations replace annually and the proportion of the different cohorts varies along the breeding season (Steinmann 2006). The reproductive period can extend from September–November (early spring) to April–May (autumn—Mills and Childs 1998). Females have a short gestation period (21 days) and each one can produce many pups in her lifetime (a maximum of 10 litters, with a mean of 6 pups per litter); juvenile females reach sexual maturity between 32 and 40 days of age and juvenile males between 35 and 39 days of age (Sommaro et al. 2009).

*Sample collection.*—*Calomys musculus* individuals were captured using traps similar to Sherman livetraps in late breeding season (April–May 2009) and in early breeding season (November–December 2010) in secondary road borders in an agricultural ecosystem located in Río Cuarto Department, Córdoba Province, Argentina (32°21'06"S, 64°20'09"W). In this linear habitat, the mean population density of *C. musculus* was  $38 \pm 3.73$  and  $258 \pm 27.43$  individuals/ha in early and late breeding seasons, respectively. The animals in this study were sacrificed by rapid cervical dislocation to support data collection for this study as well as a concurrent study on skull morphometrics. We chose cervical dislocation as euthanasia method considering the small body sizes of the studied species (Sikes et al. 2011). During dissection, the uteri from 23 females of *C. musculus* with unmistakable signs of pregnancy (12 in early breeding season and 11 in late breeding season) were removed and individual embryos were extracted. Tissue samples from each mother and embryos were preserved in 95% ethanol until DNA extraction. Trapping and handling protocols followed guidelines approved by the American Society of Mammalogists (Sikes et al. 2011). The animals were treated in a humane manner according to the current Argentinean Laws (National Law 14346).

*DNA extraction and genotyping.*—DNA was extracted using a standard salt purification procedure followed by ethanol precipitation (protocol 1 of Bruford et al. 1992). Samples were genotyped using 7 specific microsatellite loci (Cmu1, Cmu2, Cmu3, Cmu4, Cmu14, Cmu15, and Cmu17) described by Chiappero et al. (2005, 2011), following the authors' protocols and using fluorescently labeled forward primers. These loci were shown to be in linkage equilibrium in 3 natural populations from central Argentina (Chiappero et al. 2005) and in Hardy–Weinberg equilibrium in this *C. musculus* population (Chiappero et al. 2011). Amplification products were separated

on an ABI 3730XLs sequencer at Macrogen Inc. (Seoul, Republic of Korea) and allele sizes scored using Peak Scanner v1.0 (Applied Biosystems—Life Technologies Corporation, 12/2006) and MsatAllele package v1.05 in R (Alberto 2009). MsatAllele is used to visualize and bin the raw microsatellite allele size distributions. In this program, the bin limits are not fixed and are automatically defined based on the distribution of microsatellite length raw data stored in an R database. Basic estimates of genetic variability by season were obtained by calculating mean observed heterozygosity ( $H_o$ ) and mean allelic richness ( $A_r$ ) across loci using GenAlEx v.6.4 (Peakall and Smouse 2006). Estimates were computed for mothers and for offspring from early and late breeding seasons and for litters sired by 1 male or by multiple males. Levels of genetic variability were compared using a Wilcoxon test with Infostat (Balzarini et al. 2008). The occurrence and frequency of null alleles were computed in ML-Relate (Kalinowski et al. 2006) by season.

*Estimation of relatedness.*—Methods of kinship analysis fall into 2 categories: relatedness estimation (i.e., a continuous measure of overall identity by descent between individuals) or assignment of pairs or groups of individuals to categories (specific genealogical relations, like full sibs or first cousins—Blouin 2003). We used multilocus microsatellite genotypes and those 2 methods to estimate kinship relationships in *C. musculus*. For relatedness estimation among offspring in early and late breeding seasons, we excluded genetic information from all mothers to generate pairwise  $r$  values. Relatedness ( $r$ ) is a continuous measure of overall identity by descent (IBD) between individuals (Blouin 2003), being IBD the situation in which 2 alleles descended from a common ancestral allele within a population. Relatedness values range from  $-1$  to  $+1$ , with positive values indicating that 2 individuals share more alleles than expected by chance (i.e., there is some degree of relatedness) and negative values indicating that 2 individuals share fewer alleles than expected by chance. We calculated relatedness ( $r$ ) between pairs of individuals using the similarity index described by Li et al. (1993) as implemented by the program Storm (Frasier 2008). In this index, each locus was weighted using the method described by Lynch and Ritland (1999) and has been proven to be an adequate marker-based estimator of relatedness in natural populations (Van de Castele et al. 2001). Expected theoretical values predict that more related individuals will exhibit high  $r$ , whereas less related individuals will exhibit  $r$  close to or below zero (half-sibs = 0.25; full-sibs = 0.5); in genetic monogamy, it is expected higher  $r$  than in genetic promiscuity. Mean values of  $r$  were calculated within litters ( $r_{WL}$ ) and among litters ( $r_{AL}$ ) for early and late breeding seasons. In order to generate 95% confidence intervals (CIs) and to test mean differences, the observed mean  $r$  values were compared with the distribution of expected  $r$  values within and among groups of embryos sharing the same mother by season using a 1-sample  $t$ -test in Past v2.17 (Hammer et al. 2001).

*Paternity.*—In natural populations of many species, identifying and sampling putative parents is not simple or even possible (Miño et al. 2011). Taking into account that we

are studying a wild population with the absence of paternal information, the paternity of litters was assessed using 2 complementary methods. The first method, implemented in the program ML-Relate (Kalinowski et al. 2006), calculates the likelihoods of different relationship categories, unrelated (U), half-sibs (HS), full-sibs (FS), and parent-offspring (PO), between 2 individuals based on simulations and genotypic data and corrects for the presence of null alleles. The program tests the a priori interpretation of relationships, for example, if the putative relationship (i.e., full-sibs within a litter) fits the data significantly better than the alternative relationship (i.e., half-sibs within a litter, in our case, embryos that share the same mother but not the same father). We tested the significance of a given level of relationship between pairs of individuals within each group of pups sharing the same mother. Based on these data, we estimated manually the number of fathers required to explain the relationships found among pups of a litter. In the second method, the offspring were assigned to parents using Colony v2.0.5.3 (Jones and Wang 2010), a parentage and sibship assignment program that reconstructs putative sires based on a reconstructed full pedigree using a maximum-likelihood approach (Wang 2004). The major difference between this approach and that taken by other algorithms is that parentage and sibships are jointly inferred, with likelihood considered over the entire pedigree configuration rather than for pairs of individuals (Jones and Wang 2010). Offspring are first clustered into maternal families using a simulated annealing approach to maximize the group likelihood value. The clusters (sibling families) are then used to estimate the number of fathers that sired a litter and to estimate the ratios of parental contributions. As no candidate fathers are available, Colony also reconstructs paternal genotypes. In order to maximize the accuracy of Colony, we included the matrix of allelic frequencies corrected by null alleles, as estimated by ML-Relate, to avoid underestimation of the coefficient of relatedness among individuals (Kalinowski et al. 2006) and determined error rates with the MicroErrorAnalyzer v1.0 (Wang 2010). Colony was set for long-length runs using the full-likelihood method, high likelihood precision, and assuming polygamy for both males and females with maternal genotype data present. Error rates estimated by MicroErrorAnalyzer were set to 0.055 for allelic dropout and to 0.024 for genotypic error (Wagner et al. 2006; Jones and Wang 2010).

Multiple paternity ratio estimates the proportion of litters sired by more than 1 male. However, larger litters are more likely to show multiple paternity than smaller litters. Therefore, we also calculated the paternity share, which is independent of litter size (Eccard and Wolf 2009). Paternity share is an estimate of the probability that an offspring is sired by other male different from the primary male (male who sired the majority of offspring in a single litter). The confidence interval around the paternity ratio and paternity share was calculated using maximum likelihood following the method proposed by Eccard and Wolf (2009) in R v3.0.3 (R Development Core Team 2014). The observed median litter size of 5 and 6 in the early and late breeding seasons, respectively, and the empirically based

estimates of multiple paternity in litters obtained with Colony and ML-Relate were used as input. Confidence intervals were estimated by bootstrapping 100,000 times.

## RESULTS

A total of 158 individuals of *C. musculus* were genotyped, 23 mothers and 135 embryos. In early breeding season, 12 mothers had 64 offspring with an average of 5.2 pups by litter (range: 3–8). In late breeding season, 11 females had 71 offspring with an average of 6.7 pups by litter (range: 5–9). Null alleles were inferred to be present at loci Cmu2 (frequency: 6.7%) and Cmu3 (19%) in early breeding season, and Cmu17 (frequency: 16.2%), Cmu2 (11.6%), and Cmu3 (17.9%) in late breeding season. Levels of observed mean heterozygosity ( $H_O$ ) and allelic richness ( $A_R$ ) were high (Table 1). For both indices, differences were not statistically significant between mother and pups within a season (Wilcoxon test  $P > 0.05$  for both  $H_O$  and  $A_R$ ). The pups conceived in the wild in early and late breeding seasons showed similar levels of  $H_O$  and  $A_R$  (Wilcoxon test  $P > 0.05$ ). Litters with more than 1 father showed similar levels of observed heterozygosity and allelic richness as litters sired by only 1 male (Wilcoxon test  $P > 0.05$ ).

*Mean relatedness among and within litters.*—Mean values of pairwise relatedness within a litter were similar in early and late breeding seasons and were lower than expected for full sibling groups under genetic monogamy ( $r = 0.5$ ). In early breeding season, mean  $r_{WL}$  was 0.39 (95% CI = 0.36–0.43; number of pairwise comparisons = 151;  $P < 0.005$ ). In late breeding season, mean  $r_{WL}$  value was 0.38 (95% CI = 0.35–0.41;  $n = 208$ ;  $P < 0.005$ ). Mean values of pairwise relatedness among litters were very similar to theoretical expected  $r$  values

**Table 1.**—Summary of genetic variability in 7 microsatellite loci used to study paternity in *Calomys musculus* from Rio Cuarto (Cordoba, Argentina), trapped in the early breeding season (EBS: November–December 2010) and in the late breeding season (LBS: April–May 2009). Mean and standard error are shown for allelic richness ( $A_R$ ) and observed heterozygosity ( $H_O$ ).  $n$ : number of samples analyzed.

	Mother	Offspring
EBS (spring)		
$n$	12	64
$A_R$	8.434 ± 1.1	9.627 ± 1.3
$H_O$	0.773 ± 0.08	0.826 ± 0.04
LBS (autumn)		
$n$	11	71
$A_R$	6.963 ± 0.9	9.145 ± 1.25
$H_O$	0.748 ± 0.06	0.765 ± 0.05
Litters sired by 1 male		
$n$		56
$A_R$		8.827 ± 0.99
$H_O$		0.798 ± 0.05
Litters sired by more than 1 male		
$n$		79
$A_R$		9.392 ± 1.25
$H_O$		0.79 ± 0.04

for unrelated individuals; in early breeding season,  $r_{AL} = -0.03$  (95%  $CI = -0.02 - -0.04$ ;  $n = 1,898$ ) and in late breeding season,  $r_{AL} = -0.04$  (95%  $CI = -0.03 - -0.05$ ;  $n = 2,339$ ). Therefore, these results indicate that there were more half-sibs individuals within a litter than full-sibs, and this pattern did not vary between early and late breeding seasons ( $H = 0.452$ ,  $P = 0.501$ ).

**Paternity.**—Multiple paternity was estimated using 2 different methods: Colony and ML-Relate. Both methods provided unambiguous evidence that multiple mating by female corn mice is common (Table 2). However, there were some discrepancies in the estimated minimum number of potential sires by litter between the 2 methods (Table 2). ML-Relate estimated one more sire than Colony did for litters of females F3, F13, F20, and F23 (3 sires instead of 2). On the contrary, in females F16 and F18, ML-Relate estimated one less sire. However, the multiple paternity rate was altered because in F22 litter, ML-Relate assigned only 1 sire, while Colony assigned 3.

From the best inferred configuration (maximum likelihood) consisting of families obtained with Colony, we detected litters sired by more than 1 male in 13 of the 23 litters (56.5%). Similar levels of multiple paternity were found between early and late breeding seasons (proportion test with 1 tail:  $P = 0.259$ ). In early breeding season, of 12 litters, 6 (50%) had only 1 father, 4 (33.3%) had 2 fathers, and the remaining 2 litters had 3 fathers (16.7%). In late breeding season, of 11 litters, 4 (36.4%) had only 1 father, 3 (27.3%) had 2 fathers, and the remaining 4 litters had 3 fathers (36.4%; Table 2). The multiple paternity rate was estimated as 50% in early breeding season (95%  $CI = 39-62.5$ ) and 63.6% in late breeding season (95%  $CI = 52-76$ ). On the

other hand, there was no correlation between litter sizes and the number of fathers that sired a litter (Spearman's correlation  $r_s = 0.105$ ;  $P = 0.63$ ; e.g., litter sizes between 3 and 9 pups were sired by 1 male, whereas litters sizes from 4 to 8 pups were sired by 3 different males).

The paternity share estimated with Colony and ML-Relate was similar. The probability that an offspring was sired by a male different from the primary male was 13.5% in early breeding season (95%  $CI = 9.4-17.8$ ) and 15.96% in late breeding season (95%  $CI = 11.5-21.2$ ). The paternity share estimated with ML-Relate only showed a difference in late breeding season, 11.9% (95%  $CI = 8.3-15.4$ ). There was no difference between early and late breeding seasons.

## DISCUSSION

Before this study, *C. musculus* promiscuity was assumed through behavioral characteristics of males and females in relation to space use, territoriality, mating, nesting, offspring guarding, and mate discrimination (Cutrera et al. 1988; Yunes et al. 1991; Laconi and Castro-Vázquez 1998; Laconi et al. 2000; Steinmann et al. 2009; Sommaro et al. 2010b; Coda et al. 2011; Steinmann and Priotto 2011). In the absence of paternal information, relatedness coefficients and paternity patterns among pups sharing a mother were inferred based on their genotypes. The different analytical approaches implemented in this study can be seen as complementary and together yielded robustness that would not have been possible with any one method alone (Blouin 2003; Jones and Ardren 2003; Baxter et al. 2009; Jones et al. 2010; Miño et al. 2011; Martínez et al. 2013). Our

**Table 2.**—Inferred number of sires for *Calomys musculus* sibling groups using the most likely relationship estimated by Colony and ML-Relate methods. EBS: early breeding season (spring: November–December 2010); LBS: late breeding season (autumn: April–May 2009).

Sibling group				Number of sires		Sire contribution of Colony (ML-Relate)		
	Season	ID females	Litter size $n$	ML-Relate	Colony	1° Sire	2° Sire	3° Sire
EBS	F1	5	1	1	1	5 (5)		
EBS	F2	4	2	2	2	3 (3)	1 (1)	
EBS	F3	7	3	3	2	5 (5)	2 (1)	(1)
EBS	F4	4	3	3	3	2 (2)	1 (1)	1 (1)
EBS	F5	8	1	1	1	8 (8)		
EBS	F6	4	1	1	1	4 (4)		
EBS	F7	6	1	1	1	6 (6)		
EBS	F8	5	3	3	3	3 (3)	1 (1)	1 (1)
EBS	F9	5	1	1	1	5 (5)		
EBS	F10	6	2	2	2	5 (5)	1 (1)	
EBS	F11	7	2	2	2	6 (6)	1 (1)	
EBS	F12	3	1	1	1	3 (3)		
LBS	F13	9	3	3	2	7 (6)	2 (2)	(1)
LBS	F14	5	1	1	1	5 (5)		
LBS	F15	9	1	1	1	9 (9)		
LBS	F16	7	2	3	3	4 (6)	2 (1)	1
LBS	F17	6	1	1	1	6 (6)		
LBS	F18	6	2	3	3	4 (4)	1 (2)	1
LBS	F19	5	3	3	3	3 (3)	1 (1)	1 (1)
LBS	F20	7	3	2	2	5 (5)	2 (1)	(1)
LBS	F21	5	1	1	1	5 (5)		
LBS	F22	8	1	3	3	4 (8)	3	1
LBS	F23	4	3	2	2	3 (2)	1 (1)	(1)

study provides clear evidence of multiple paternity in free-living corn mice based on genetic data. In this study, 56.5% (13 out of 23) of the litters were multiply sired. According to Dean et al. (2006), Bryja et al. (2008), Firman and Simmons (2008a, 2008b), and Borkowska et al. (2009), percent values of multiple-sired litters obtained in our study provide direct evidence of multiple paternity in single litters of voles and mice. Thus, our results are consistent with the previous classification of the *C. musculus* mating system.

In promiscuous voles and mice, multimale mating occurs more frequently in the late breeding season (high density) than in the early breeding season (low density—Clutton-Brock 1989; Davies 1991; Waterman 2007). Bryja et al. (2008) found that multiple paternity increased visibly with high abundance in 4 promiscuous *Apodemus* mice species. However, in this study, the multiple paternity rate between early and late breeding seasons was similar. According to Bond and Wolff (1999) and Ishibashi and Saitoh (2008), the 2 main factors that should limit reproductive success of male rodents are the number of females to which they have access and the number of male competitors with which they interact. Thus, variations in mating behavior are expected both within and between populations as a consequence of the adaptive adjustment of male and female behavior to differences in the social and ecological local environment (Emlen and Oring 1977; Clutton-Brock and Harvey 1978; Clutton-Brock 1989; Loughran 2007). Sommaro et al. (2010b) found that *C. musculus* females maintain their territories regardless of population density values and that at high population density they limit the size of the breeding population by constraining other females from settling. On the other hand, the upper limit in the number of *C. musculus* breeding males is reached through induced territoriality derived from intrasexual social restrictions (Sommaro et al. 2010b; Steinmann and Priotto 2011). Thus, *C. musculus* spacing behavior would explain the similarity between multiple paternity rates at low and high population density found in this study.

However, if females can obtain enough sperm to fertilize all her ova from a single insemination (Simmons 2005; Parker and Birkhead 2013), why then, given the involved costs (Madsen 2011; Ashby and Gupta 2013), do females copulate with more than 1 male? In recent years, there is growing evidence that females of both invertebrate and vertebrate species can benefit from mating with several males over a single reproductive cycle, even when this mating strategy involves significant costs for females (Zeh and Zeh 2001; Hosken and Stockley 2003; Zhang et al. 2004; Simmons 2005; McFarlane et al. 2011; Kvarnemo and Simmons 2013). The idea that females can benefit from mating with multiple males has led to widespread research of this issue, thus multimale mating has so far been reported in more than 130 species of mammals (Wolff and Macdonald 2004). In voles and mice, given multimale mating potential costs, this mating strategy should compensate females that mate with several males (Klemme et al. 2006, 2007; Bryja et al. 2008; Firman and Simmons 2008a, 2008b; Thonhauser et al. 2013). However, although females often show multimale mating, the adaptive functions remain unclear.

*Litter size.*—In female house mice (*Mus musculus musculus*—Thonhauser et al. 2013), Utah prairie dogs (*Cynomys parvidens*), and Gunnison's prairie dogs (*Cynomys gunnisoni*—Hoogland 1998, 2013), multimale mating is responsible for larger litter size and higher conception rates. However, Wolff and Macdonald (2004) proposed that this benefit is not widely present in mammals, and Keil et al. (1999), Wolff and Dunlap (2002), and Stockley (2003) provided evidence that mating with several males does not increase litter size in promiscuous rodents. Our results indicated that mean litter size did not vary between monandrous and polyandrous *C. musculus* females. According to Humphries and Boutin (2000), McAdam et al. (2002), and Réale et al. (2003), exceeding optimal litter size can have long-term fitness consequences both for offspring growth and survival and/or female survival and future reproductive success. These authors proposed that these associated costs may only be avoided in species with biparental or communal offspring care, 2 behavioral features absent in *C. musculus*. On the other hand, even in the context of sigmodontine species, *C. musculus* is a highly prolific species (Buzio and Castro-Vásquez 2002). The corn mouse owes part of its remarkable reproductive capability to an early genital maturation: males and females reach sexual maturity at an early age (30 days old—Buzio and Castro-Vásquez 2002; Sommaro et al. 2009). Moreover, Buzio and Castro-Vásquez (2002) registered ovulation in recently weaned females (close to 20 days of age). In addition, during the reproductive period, females give birth to multiple litters, with an average litter size of 6 pups, and show a high frequency of postpartum estrus, which implies that a new pregnancy may overlap with the lactation of the previously produced litter (Busch et al. 2000; Sommaro et al. 2009). Therefore, the costs (in terms of time and energy expenditure, injuries, and sexually transmitted diseases) of mating with several males in order to increase litter size would not be justified in *C. musculus* females.

*Genetic variability.*—Female reproductive success can also depend on the number of mates because of its effect on genetic variability within broods (Kvarnemo and Simmons 2013). This genetic variability is much lower in litters produced by 2 parents than in litters produced by 3 or more contributors; in the small marsupial *A. agilis* (Kraaijeveld-Smit et al. 2002), and in prairie dog species *Cynomys ludovicianus* and *C. leucurus* (Chesser 1983; McCullough 1991; Daley 1992), multimale mating was found to enhance offspring genetic variability. In a nonresource-based mating system like the corn mouse, multimale mating would occur as a trading-up strategy to improve genetic variability of the offspring. Increasing genetic variability within litters can increase the survival probability in habitats that experience unpredictable changes from one generation to the next (Yasui 1998; Crean and Marshall 2009). *Calomys musculus* is a good colonizer of habitats with high temporal heterogeneity such as agroecosystems (Bilenca and Kravetz 1995; Busch et al. 2000; Sommaro et al. 2010b). According to Pardiñas et al. (2000, 2004), agricultural expansion and intensification may have favored both dispersal and abundance of generalist and opportunistic species such as *C. musculus*. Our results showed

that in *C. musculinus*, litters with more than 1 father showed as much genetic variability as litters with a single sire. Considering the wide range of habitats used by *C. musculinus*, the potential role of multiple mating for the increase of genetic variability of the population should not be discarded hastily in this species. Although some authors (reviewed in Karl 2008) consider that multiple paternity would reduce the effective population size and decrease the genetic variability of the population (because each mating may result in fewer offspring per male than the expected in a genetic monogamy), others consider that multiple paternity may increase the effective population size and genetic variability of the population (Pearse and Anderson 2009). Due to the fact that the increase in genetic variability represents a long-term benefit, studies comprising longer time spans (generations) than this one must be carried out to elucidate this issue.

This study is the first to show that multiple paternity occurs in *C. musculinus* litters in a wild population. The possible adaptive significance of multimale mating discussed in our study could not be applied in *C. musculinus*; litter size and genetic variability within broods did not vary between monandrous and polyandrous females. Based on the absence of infanticide by siring males, and the low offspring survival in the presence of nonsiring males, Coda et al. (2011) proposed that *C. musculinus* females would mate with multiple males as a counter-strategy against infanticide by males. In promiscuous voles and mice, where infanticide is widespread, Wolff and Dunlap (2002) and Wolff and Macdonald (2004) proposed that by mating with several males during the regular period of estrus, females would confuse paternity of their litters and persuade males to tolerate their young once born. Klemme and Ylönen (2010) found that polyandry enhances offspring survival in female bank voles *Myodes glareolus*. This possible benefit of multimale mating in *C. musculinus* is not faulty in its logic and has a strong empirical support. However, experimental studies of the benefits of genetic promiscuity in both female individuals and the population would be a very important task for future research, where *C. musculinus* may be a suitable model species.

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