

## INBREEDING IN THE GREATER WHITE-TOOTHED SHREW, *CROCIDURA RUSSULA*

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**Abstract.**—We combined mark-and-recapture studies with genetic techniques of parentage assignment to evaluate the interactions between mating, dispersal, and inbreeding, in a free-ranging population of *Crocidura russula*. We found a pattern of limited and female-biased dispersal, followed by random mating within individual neighborhoods. This results in significant inbreeding at the population level: mating among relatives occurs more often than random, and  $F_{IT}$  analyses reveal significant deficits in heterozygotes. However, related mating partners were not less fecund, and inbred offspring had no lower lifetime reproductive output. Power analyses show these negative results to be quite robust. Absence of phenotypic evidence of inbreeding depression might result from a history of purging: local populations are small and undergo disequilibrium gene dynamics. Dispersal is likely caused by local saturation and (re)colonization of empty breeding sites, rather than inbreeding avoidance.

**Key words.**— $F$ -statistics, inbreeding depression, kin recognition, mate choice, sex-biased dispersal.

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Because natural populations are finite in size, mating sometimes occurs among relatives, even when partners meet at random. Offspring born from such matings are said to be inbred, and they often display phenotypic abnormalities resulting in a loss of fitness through lower viability or fertility. This inbreeding depression has been repeatedly documented in a number of natural populations, including invertebrates (Chen 1993; Saccheri et al. 1998), reptiles (Madsen et al. 1996; Olsson et al. 1996), birds (Greenwood and Harvey 1978; Bensch et al. 1994; Keller et al. 1994; Brown and Brown 1998; Keller 1998; Westemeier et al. 1998), and mammals (Jimenez et al. 1994; Coltman et al. 1998; Coulson et al. 1998; see reviews by Lynch and Walsh 1998; Crnokrak and Roff 1999; Keller and Waller 2002). Inbreeding depression often appears to be stronger in the field than in the laboratory (presumably because the mild conditions prevailing in the latter alleviate the burden of deleterious alleles) and, in a few cases, has been shown to threaten the persistence of small populations (Saccheri et al. 1998; Madsen et al. 1999; see reviews by Hedrick and Kalinowski 2000; Keller and Waller 2002).

Although the detrimental role of inbreeding seems to have approached the status of law, it does not always need to be so, as evidenced by the absence of depression in a few well-documented cases (e.g., Gibbs and Grant 1989; Reeve et al. 1990; Keane et al. 1996). This may have arisen from the purging of genetic load through recurrent inbreeding events. When costs are low, natural selection may actually favor inbreeding, for at least two reasons. First, outbreeding may dismantle genetic coadaptations built up locally through linked gene complexes (Shields 1982, 1983; Bateson 1983; Templeton 1986; Wiener and Feldman 1993). Second, inbred matings bring direct benefits to males (and inclusive benefits to females through increased reproductive output of related males), as long as they do not forfeit other breeding opportunities (Parker 1979, 1983; Smith 1979; Waser et al. 1986).

In the general case, however, inbreeding costs appear to be important enough that ways to avoid them have evolved.

Indeed, incestuous matings (defined as parent-offspring or sibling-sibling pairing) are rare in the field (normally less than 2% according to Ralls et al. 1986; see also Harvey and Ralls 1986), which, in many instances, could not be achieved without some inbreeding-avoidance mechanisms. One such mechanism consists of choosing mates according to kinship-related cues (Ralls et al. 1986; Andersson 1994; Pusey and Wolf 1996; Bull and Cooper 1999). Familiarity-based recognition, seemingly the most widespread kin-discrimination mechanism in higher vertebrates (Berger et al. 1997; Komdeur and Hatchwell 1999), certainly limits matings among full- or half-sibs from the same brood. It does not, however, prevent mating among siblings from successive broods or among paternal half-sibs.

An alternative way to avoid inbreeding is to disperse (Ralls et al. 1986; Johnson and Gaines 1990; Andersson 1994; Pusey and Wolf 1996). The prevalence of sex biases in dispersal has been invoked as evidence for its pervasive function of inbreeding avoidance (Pusey 1987). However, such biases can also result from other causes. The widespread male bias in dispersal among polygynous mammals (Greenwood 1980; Dobson 1982) seems best explained by asymmetric kin competition pressures: in female-defense systems, local mate competition on males normally exceeds local resource competition on females (Perrin and Mazalov 2000). Some exceptions to the general mammalian pattern, by contrast, seem better explained by inbreeding-avoidance arguments: several cases of female-biased dispersal in mammals correspond to situations in which one or a few males monopolize local reproduction over a time period longer than the maturation time of their daughters (Clutton-Brock 1989).

This latter hypothesis was actually invoked (Balloux et al. 1998) to account for the female-biased dispersal in the shrew *Crocidura russula*. The unusual breeding system of this annual species typically consists of monogamous pairs defending a common breeding territory, where they rear up to four litters from March to September (Cantoni and Vogel 1989). Natal dispersal is mostly observed in female weanlings from the first litter (born in March), who normally reproduce the same year they are born (Favre et al. 1997). Females from successive litters usually settle for winter in the parental ter-

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ritory or the immediate vicinity and only reproduce the following breeding season, at which time their father will normally have died. By dispersing, first-litter females may thus avoid fertilization by their fathers or brothers. However, three points remain:

(1) About half of the females and most of the males settle locally, which creates a high potential for inbreeding in the following breeding season.

(2) Dispersal distance is low, as shown by the significant correlation between genetic and geographic distances over a few kilometers (Balloux et al. 1998); thus, even dispersing individuals may mate with relatives.

(3) How philopatry translates into actual inbreeding is unclear, without information on possible kin discrimination mechanisms and behavioral avoidance, if any.

The present work is an attempt at evaluating, in a free-ranging population of *Crocidura russula*, whether inbreeding avoidance is likely to be a driving force behind mating and dispersal patterns. To do so, we evaluated actual inbreeding by measuring the relatedness among mating partners, estimated inbreeding avoidance by comparing this relatedness with that among potential mating partners, and measured inbreeding depression in terms of both fecundity loss of inbred matings and subsequent survival and reproductive success of inbred offspring.

MATERIALS AND METHODS

Field Sampling

*Crocidura russula* is a small (11–14 g) insectivorous mammal, widespread in southern and central Europe. The species is anthropophilic in the northern part of its distribution (including the study area), where it lives in discrete populations, inhabiting villages and suburbs. Thermally favorable sites (farms, stables, compost piles) are required to meet the energetic needs of the cold season (Genoud and Hausser 1979). Winter metabolic demands are further lowered by communal nesting and daily bouts of torpor (Genoud 1985), but winter resource shortage remains the most important source of mortality and may temporally wipe out local populations (Genoud and Hausser 1979).

Our study site is an area of about 250 m × 350 m on the campus of the University of Lausanne (Switzerland) at Dorigny (6°34'E, 45°31'N, 400 m above sea level). A total of 180 Longworth (Penlon Ltd., Abingdon, England) traps, baited with *Tenebrio molitor* larvae, were settled in autumn 1997 at all potentially favorable breeding sites, and one overnight trapping session was performed once a week through summer 2002. Because female weight drops from about 20 g to 14 g at parturition, weekly trapping allowed quite precise estimation of the dates of birth of juveniles in most cases. Weanlings leave the nest for short periods and go on limited exploratory excursions from day 13 to day 20 after birth (when weaning is normally achieved; Genoud and Vogel 1990). Weanlings were often captured during this period within the parental territory. The present study focuses on the cohorts born in 1998 and 1999 (Fig. 1), as well as their parents (some of which born in 1997) and direct offspring (some of them born in 2000). All individuals caught were weighed and marked by toe clipping, their sex and breeding status were

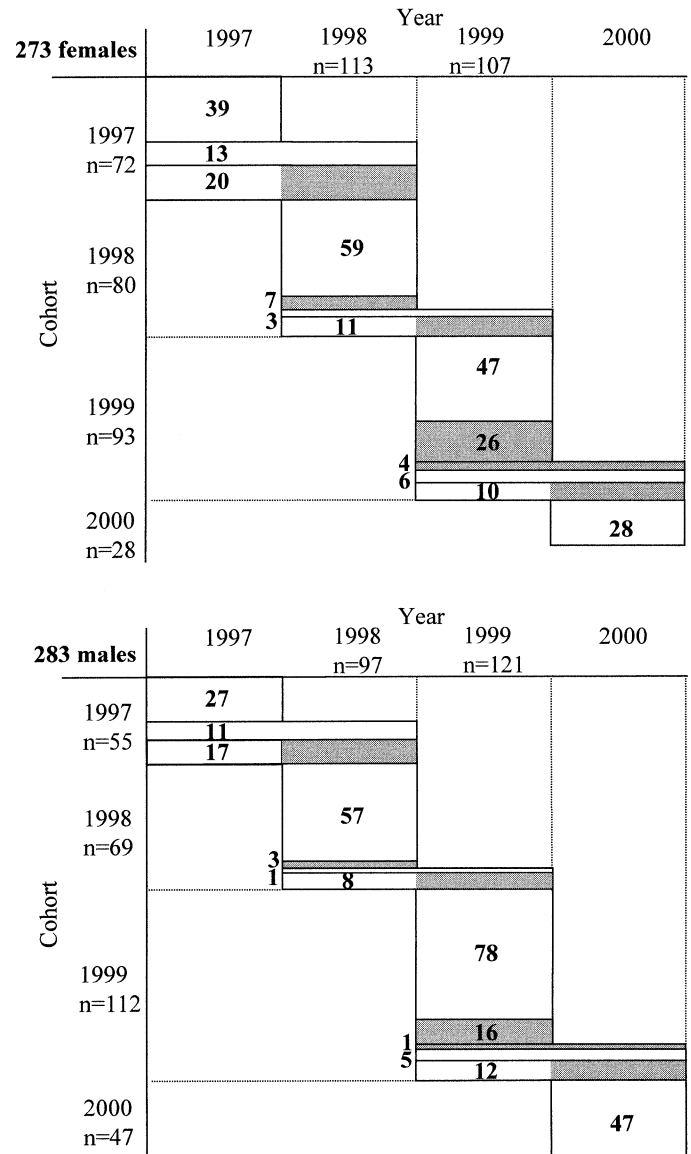


FIG. 1. Distribution by sex, year, and reproductive status of the 556 individuals considered in the present study. The top panel is for females, and the bottom panel for males. Shaded areas represent reproducing individuals. For example, the 93 females from the 1999 cohort consisted of 47 individuals that failed both to reproduce and to survive winter, 26 that reproduced in 1999 and then died, four that reproduced both in 1999 and 2000, six that survived to 2000 but did not reproduce, and 10 that survived to 2000 and reproduced only that year. The reproductive status of the 1997 cohort was not known for this year. For the 2000 cohort, only locally born individuals are represented.

determined (juveniles are grayish and lighter than adults, breeding males present visible lateral glands, and breeding females have visible teats). Toes were kept frozen (−20°C) before DNA extraction.

Genetic Analyses

DNA was extracted using either the standard method of Sambrook et al. (1989) or the salting-out procedure of Miller et al. (1988). All individuals were scored for 12 microsatellite

loci, including loci 9, 17, 23, 45, 53, 54, 57, and 72 of Favre and Balloux (1997) as well as loci 24, 41b, 49, and 52 designed for the present study (GenBank access numbers AY034426, AY034427, AY034428, and AY034429, respectively). Allele number per locus ranged from three to 16, average 9.8 (effective number 5.4). The expected heterozygosity was calculated as

$$H_T = \frac{1}{mn} \sum_{j>i}^m \sum H_{ij}, \quad (1)$$

where  $m$  is the number of loci,  $n$  the number of individuals in the population, and  $H_{ij}$  the expected number of heterozygotes for the alleles  $i$  and  $j$ , calculated from their frequencies  $p_i$  and  $p_j$  as

$$H_{ij} = \frac{4n^2 p_i p_j}{2n - 1} \quad (2)$$

(Nei 1987). The relative deficit in heterozygotes ( $F_{IT}$ ) was estimated and tested using the software FSTAT (Goudet 1995). Relatedness values among individuals were calculated with KINSHIP 1.2 (Goodnight and Queller 1999), using all individuals caught during the year considered as the reference population.

#### Inbreeding Avoidance

Inbreeding avoidance was tested by checking whether the relatedness of focal individuals with their actual mates differed statistically from that with potential partners available at the time of mating. In a first step, we considered as potential partners all adults of the opposite sex present in the study area during the month in which the focal individual paired with its partner. In a second step, we restricted the spatial scale of the analysis to individual neighborhoods by considering as potential partners only opposite-sex adults captured within 50 m from the focal individual. This value corresponds to the average maximal distance between two traps in which a given individual was caught within a given month.

#### Parentage Assignments

Individual fecundities were calculated through parental assignment using the software PROBMAX (Danzman 1997). Paternity and maternity were assessed independently for all juveniles caught within the study area, allowing for one mismatch. All adults captured within the area during the year of interest were considered as potential parents in the parent-hood exclusion analyses. In all cases where more than one male or one female genotype could not be excluded as parents, temporal and spatial information allowed us to complete the exclusion process unambiguously. Indeed, in all of these cases, one of the genetically compatible mothers (or fathers) was actually paired with the assigned opposite-sex parent, while the other was either dead or spatially isolated from the assigned opposite-sex parent.

The correctness of assignments was further tested by comparing the regression of offspring heterozygosity ( $H_i$ ) on the relatedness ( $r_i$ ) among their putative parents, with the expected relationship (Appendix):

$$H_i = H_T - r_i \left( H_T - \frac{H_o}{2} \right), \quad (3)$$

where  $H_T$  is the expected heterozygosity and  $H_o$  is the observed heterozygosity.

#### Inbreeding Depression

Because fecundities were not normally distributed, we used a nonparametric correlation (Kendall's  $\tau$ ) between the number of successfully weaned offspring and the relatedness coefficient among their parents to test whether related partners suffered from a depressed fecundity. The possible fitness loss of inbred offspring was also investigated along two lines. First, we evaluated whether inbred offspring suffered from a lowered access to reproductive status by comparing the average heterozygosity of individuals that successfully mated with that of individuals that did not (either because they disappeared during winter or because they did not obtain a mating partner during the breeding season). Second, for those individuals that reproduced, we estimated the fecundity loss of inbred individuals from a nonparametric correlation (Kendall's  $\tau$ ) between their reproductive success (number of offspring successfully weaned) and their heterozygosity.

Power analyses were conducted by bootstrapping the actual distributions and simulating different effects and sample sizes. We calculated both the effect required (given our sample size) and the sample size required (given the observed effect) to reach a 0.8 power (80% of simulated sets that differed at the 0.05 level from the null hypothesis). In every case at least 500 replicates were performed. All statistical analyses were conducted using S+ 2000 (MathSoft, Inc., Seattle, WA).

## RESULTS

#### Inbreeding Avoidance

A total of 556 individuals (273 females, 283 males) were included in the analyses (Fig. 1). Observed heterozygosity values were extremely variable among individuals (ranging from 0.083 to 1.000), but averages were high and quite similar between the two years of study ( $H_o$  0.735 and 0.738, respectively). Expected heterozygosity values ( $H_T$ ) were consistently higher (0.794 and 0.776, respectively), which resulted in significant  $F_{IT}$ -values (0.074 and 0.049,  $P < 0.001$  for both years).

A total of 68 females and 45 males reproduced in 1998 or 1999 (Fig. 1). Three females reared offspring from unidentified males, having been fertilized before settling in the study area. The other individuals formed 81 pairs, which corresponds to an average of 1.8 partners per male and 1.2 partners per female. These values, in line with those (1.5 and 1.1, respectively) obtained by Bouteiller and Perrin (2000), confirm that monogamy is far from being strict in this species (although polyandry appears rarer than polygyny).

The estimated relatedness values among mating partners in these 81 pairs were highly variable (ranging from  $-0.274$  to  $0.756$ ), with an average value of  $0.075$ , marginally exceeding zero ( $P = 0.05$ , one-sided Wilcoxon signed-rank test). The distribution is right-skewed (Fig. 2), with values exceeding  $0.25$  in 17 pairs (21%) and  $0.50$  in seven pairs (8–9%). This later threshold corresponds to the expected relat-

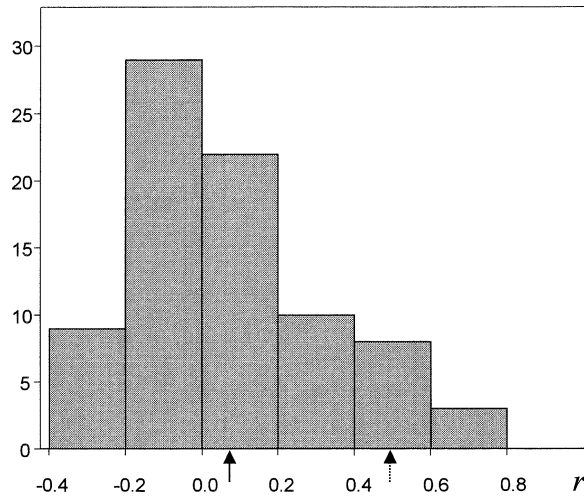


FIG. 2. The relatedness values ( $r$ ) among mating partners in 81 pairs are widely distributed around a positive average value (0.075; plain arrow). The distribution displays a right skew, with about 17% of matings among close relatives (values centered on 0.5, dashed arrow)

edness among parent-offspring or full-sibs (i.e., close inbreeding). Indeed, analysis of 46 full-sibships from the 1998 and 1999 cohorts provided an average relatedness of 0.499. Values were symmetrically distributed with standard deviation 0.126 (range = 0.208–0.815). From this distribution, the proportion of close inbreeding (full-sib level) can be estimated to be approximately 17% (i.e., twice the number of values exceeding the 0.5 threshold). Pedigree analyses revealed in 1999 a minimum of six pairings among close relatives (of 46), including cases of pairings among father-daughter, mother-son, full-sibs (from the same litter), and half-sibs. In one case, a male first mated with his half-sister, then with a daughter born from this mating. He then successfully reared two more litters with his half-sister and three with his daughter.

Individuals were significantly more related to their actual partner than to potential partners from the whole population (females:  $0.067 \pm 0.026$  [SE] vs.  $-0.012 \pm 0.008$ ,  $n = 65$ ,  $P < 0.05$ ; males:  $0.091 \pm 0.034$  vs.  $-0.005 \pm 0.007$ ,  $n = 45$ ,  $P = 0.05$ , Wilcoxon signed-rank test). When only potential partners present in local neighborhoods were considered, actual partners were still slightly more related than by chance, although the difference was not significant (females:  $0.064 \pm 0.026$  vs.  $0.023 \pm 0.018$ ,  $n = 55$ ,  $P = 0.32$ ; males:  $0.077 \pm 0.038$  vs.  $0.045 \pm 0.020$ ,  $n = 40$ ,  $P = 0.54$ ; Wilcoxon signed-rank test). Five males and 10 females had no potential partner in their neighborhood besides their actual mate and so were not included in this latter analysis. For the individuals included, the number of potential partners besides their actual partner ranged from one to 15 for both sexes. Given our sample size and observed distributions, the difference in relatedness required to reach a 0.8 power was close to 0.1 for both sexes, and the sample sizes required to get the same power, given the observed effects, exceeded 400 for females and 800 for males.

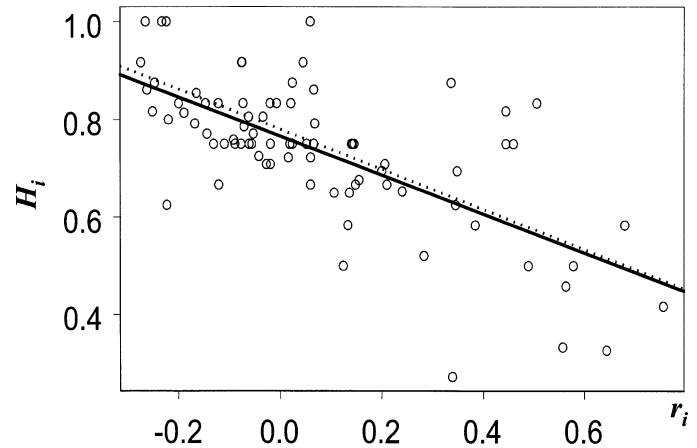


FIG. 3. The observed regression (bold line) of offspring heterozygosity ( $H_i$ ) on the relatedness ( $r_i$ ) among their putative parents is extremely close to the relation expected were the parents correctly assigned (dashed thin line).

#### Parentage Assignments

The large amount of genetic variance allowed parental assignment analyses to reach an exclusion probability of 0.998 (Jamieson 1994). Of 354 offspring typed (the 1998 and 1999 cohorts), a significantly larger proportion of females (69/173, 40%) than males (33/181, 18%) could not be assigned to at least one parent ( $\chi^2 = 18.07$ ,  $P < 0.001$ ), thereby confirming the female-bias in dispersal. A total of 252 offspring could thus be attributed (with the help of spatial and temporal information for 60 of them) to the 81 mating pairs, which corresponds to  $3.11 \pm 3.05$  (SD) offspring per pair. The variance in fecundity was thus largely in excess of a Poisson distribution and the range quite broad (one to 17).

The regression of mean offspring heterozygosity on the relatedness among their putative parents ( $H_i = 0.77 - 0.40r_i$ ;  $P < 0.0001$ ;  $r^2 = 0.46$ ) is extremely close to the theoretical expectation ( $H_i = 0.78 - 0.41r_i$ ), obtained by substituting  $H_T$  (0.78) and  $H_o$  (0.74) in equation (3). The two relations are basically indistinguishable (Fig. 3). This impressive fit provides strong confidence in the assignments made; furthermore, it shows that individual heterozygosity correctly reflects the relatedness among their parents, even though the residual sampling variance remains large.

#### Inbreeding Depression

The fecundity of mating pairs was totally uncorrelated with the relatedness among partners (Fig. 4a; Kendall's  $\tau = 0.019$ ,  $P = 0.798$ ,  $n = 81$ ). Given our sample size, a regression coefficient close to  $-0.35$ , corresponding to a 16% decline in fecundity for a full-sib mating ( $r = 0.5$ ) relative to an outbred mating ( $r = 0.0$ ), was required to reach a 0.8 power. The sample size required to reach this power (given the effect observed) was close to 10,000.

From the 354 juveniles of the 1998 and 1999 cohorts, 58 females (of 173) reached reproductive status, 37 the year they were born and 21 the following year. As for males, 40 individuals (of 181) reproduced, 20 the year they were born, and 20 the following year. Four females and one male from the

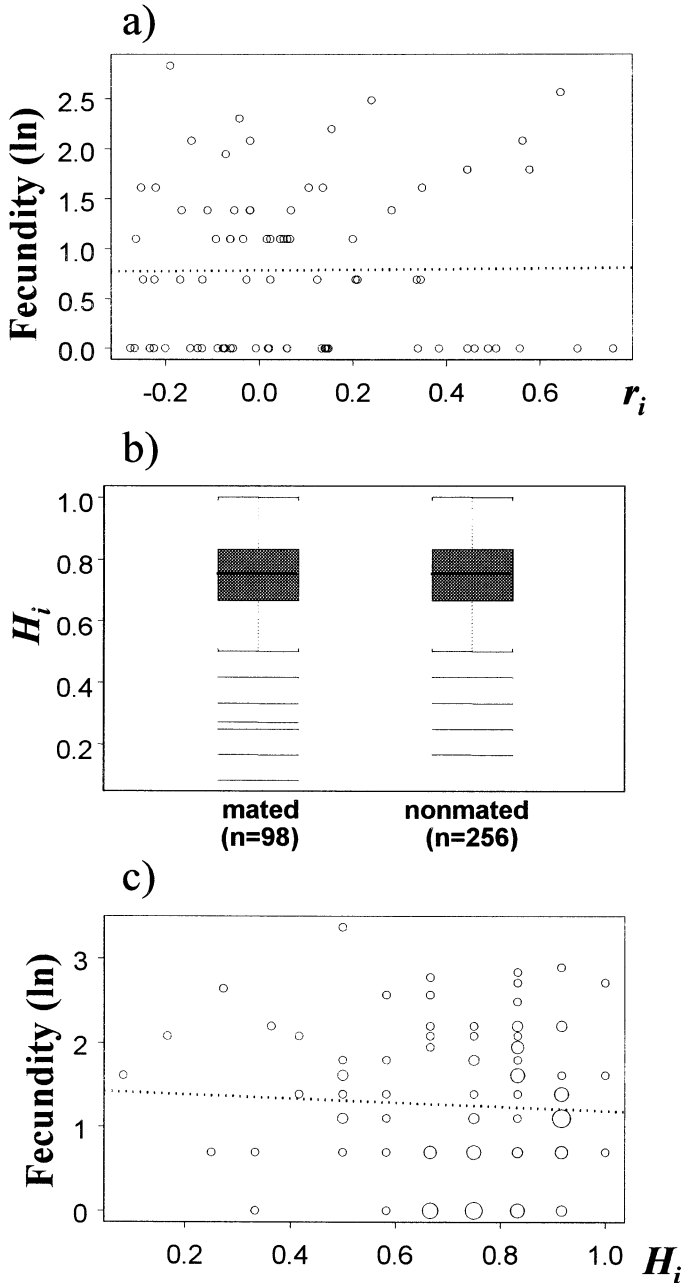


FIG. 4. (a) The relatedness among mating partners ( $r_i$ ) had no effect on their fecundity ( $\ln$  transformed). A linear regression (dotted line) explains 0.0001 of the variance. (b) The offspring that successfully mated were not less inbred than those that did not mate. Boxes represent medians and the two first quartiles. Horizontal lines are outliers (more than 1.5 times the interquartile range from the median). (c) Individual heterozygosity ( $H_i$ ) had no detectable effect on reproductive output.

1999 cohort managed to reproduce in two successive years (Fig. 1). Winter mortality was the main reason for reproductive failure, because 106 females and 135 males disappeared in winter without having reproduced, whereas only nine females and six males reached the breeding season but failed then to breed successfully. The probability of attaining a reproductive status was unaffected by inbreeding level (Fig. 4b): successful breed-

ers had an average heterozygosity of  $0.728 \pm 0.192$  ( $n = 98$ ), as compared to  $0.732 \pm 0.162$  ( $n = 256$ ) for those individuals that did not, a nonsignificant difference (Kruskal-Wallis test,  $\chi^2 = 0.275$ ,  $P = 0.60$ ). Given our sample size and observed distributions, a difference of 0.04 (a minute fraction of the observed heterozygosity range) was required to reach a 0.8 power, and the sample size needed to reach this level, given the difference noted, exceeded 10,000.

Finally, the fecundity of these 98 individuals that reached reproductive status was totally uncorrelated with their heterozygosity (Kendall's  $\tau = -0.0002$ ,  $P = 0.998$ ; Fig. 4c). Given our sample size, a regression coefficient of 0.3 (i.e., 0.3 lethal equivalent per gamete), corresponding to a 6% fecundity decline for offspring born from a full-sib mating ( $F = 0.425$ ) relative to an outbred mating ( $F = 0.22$ ), was required to reach the 0.8 power level. For the observed effect, the power of the test was close to 0.05 for any sample size.

#### DISCUSSION

Our results provide no indication of either inbreeding avoidance or inbreeding depression in *C. russula*. The pattern emerging is one of limited and female-biased dispersal, followed by random mating within neighborhoods. As already documented by Favre et al. (1997) and Balloux et al. (1998), a large majority of males stay within the parental vicinity, while about half of the females disperse. After dispersal, as our present results show, mating is essentially random within individual neighborhoods, without any indication of avoidance or preference of related partners.

Pedigree analyses revealed several cases of close inbreeding, some among individuals who previously had the opportunity to build a familiarity-based recognition, such as mother-son, full-sibs from the same litter, or father-daughter (males share family nests in *C. russula*; Cantoni and Vogel 1989). The frequency of matings among close relatives documented here (estimated to be 17%) largely exceeds the average figure of 2% or less previously reported for mammals (Harvey and Ralls 1986; Ralls et al. 1986). Absence of inbreeding avoidance is further supported by the random relatedness among partners within neighborhoods. At this local scale, breeding adults were not less related to their actual mate than to other potential mates.

At the population scale, relatedness with actual mates exceeded that with potential partners. This result, together with the positive  $F_{IT}$ -values, might be taken as evidence for preferential mating among relatives. We argue, however, that these patterns arise merely as the necessary consequences of limited dispersal. As most males and part of the females remain within their parental neighborhoods, mating statistically occurs more often among relatives when considered at the population level, even in complete absence of local mate choice.

Close inbreeding would obviously still be more frequent in complete absence of dispersal, so the point could be made that dispersal evolved to avoid inbreeding (Pärt 1996). This point is correct in principle, but, in the present instance, absence of inbreeding depression argues against inbreeding avoidance as a significant influence on dispersal. Relatedness among partners, although highly variable, had no detectable effect on their fecundity. Offspring homozygosity, shown

here to reflect parental relatedness, had no more effect on their access to reproduction or ensuing fecundity.

Hedrick and Kalinowski (2000) pointed out that inbreeding depression may pass undetected if examined in captive situations; if only one or a few components of fitness are monitored; or if analyses lack statistical power, either because sample size was too small or inbreeding coefficients not variable enough. None of these caveats apply to our study because we examined a field population and monitored fitness itself (i.e., lifetime reproductive output), not a surrogate. As far as power is concerned, inbreeding level was highly variable (both in terms of relatedness among parents and offspring heterozygosity), and our sample size was sufficient to detect 0.3 lethal equivalents per gamete. Considerably higher inbreeding loads (up to 20 times higher; Jimenez et al. 1994) have been documented in natural populations (Keller and Waller 2002). Absence of inbreeding depression is further supported in the present instance by the huge sample size (exceeding 10,000) that would be required to have an 80% probability of disentangling the observed effect from measurement error or chance.

Another important prerequisite is that relatedness and inbreeding coefficients were correctly assessed. Absence of inbreeding avoidance or depression has been claimed from studies where pedigrees had been established on the basis of social partnerships only (e.g., Van Noordwijk and Scharloo 1981; Rowley et al. 1986; Craig and Jamieson 1988; Gibbs and Grant 1989). In at least one case (the fairy wren, *Malurus splendens*), subsequent genetic analyses have shown that females are seeking extrapair copulations from unrelated males, who actually sire 65% or more of the offspring (Brooker et al. 1990). In our study, pedigrees, relatedness, and inbreeding coefficient were established on the basis of genetic markers, which allowed us to verify that social partners were indeed the genetic contributors.

Our failure to observe an effect of inbreeding is not unprecedented: Keane et al. (1996), for instance, could not find any sign of inbreeding avoidance or inbreeding depression in a field study of dwarf mongooses (*Helogale parvula*), even though they also assigned parentages on the basis of genetic markers.

These negative results obviously pose a series of questions, the first of which is what causes the female-biased dispersal in *C. russula*? Besides inbreeding avoidance, the main selective influences on dispersal are kin competition avoidance, kin cooperation, and spatio-temporal variations in habitat saturation (Gandon and Michalakis 2001; Perrin and Goudet 2001). Theoretical models (Perrin and Mazalov 2000) show that, in stable habitats and absence of inbreeding depression, a female-biased dispersal is expected if females suffer more than males from local competition. In monogamous species, where males play a significant role in acquiring and defending resources, local-mate and local-resource competition exert similar selective pressures on both sexes, so that unbiased dispersal is expected (as otherwise supported by empirical patterns in mammals; Dobson 1982). In the present instance, males have been shown to display some polygyny and to benefit from it (Bouteiller and Perrin 2000), which should increase local mate competition and thereby favor male dispersal. However, males apparently play an important role in territory acquisition and defense (Cantoni and Vogel 1989). If this task is made easier by familiarity with the

natal area (Greenwood 1980; Pärt 1994; Bensch et al. 1998) or by living among related neighbors (e.g., Watson et al. 1994; Koprowski 1996), then male philopatry should evolve (Perrin and Goudet 2001).

Disequilibrium population dynamics might also induce a sex bias in dispersal. *Crocidura russula* lives in small and highly structured breeding groups (Balloux et al. 1998). Suitable breeding sites are regularly vacated and opened for colonization by heavy winter mortality (Genoud and Hausser 1979). Maturing young females should thus be under selection to disperse whenever parental neighborhoods are saturated. The present study moreover showed that several females were already fertilized when settling within our study area. Thus, some females may mate while still in the process of dispersing and already be pregnant when reaching open territories. Experimental studies have shown that one pregnant female suffices to colonize an empty site (Vogel 1999).

The second main question to be asked concerns the lack of inbreeding depression. This points to a history of close inbreeding that might also partly result from disequilibrium gene dynamics. Whenever empty sites are colonized by one pregnant female or one founding pair, then close inbreeding necessarily follows. In such a case, the benefits of successful colonization largely outweigh the possible costs of inbreeding depression. Even when it does not decimate local demes, winter mortality creates bottlenecks, in such a way that the only partners available in spring may turn out to be relatives. In the present study, 17 individuals (of 110, 15%) had but a single potential partner within their individual neighborhood. Furthermore, the scarcity of favorable sites, as well as the costs of dispersing among them, may induce offspring of both sexes to remain within parental neighborhoods to inherit good breeding and overwintering sites. Over the long term, recurrent inbreeding may largely purge natural populations from deleterious alleles (even though the process certainly takes many generations; Brewer et al. 1990; Ballou 1997). Such a purging is regularly invoked whenever populations have been strongly structured in small demes for long periods of time (e.g., Gibbs and Grant 1989; Reeve et al. 1990; Keane et al. 1996), but has yet to be demonstrated in nature. Furthermore, we find hard to understand how this proposed mechanism may lead to a significant purging of deleterious alleles, but still maintain the high level of genetic diversity observed at neutral markers.

Finally, why don't shrews mate more consistently with relatives, given the absence of genetic load? Polygyny enhances male fitness (Bouteiller and Perrin 2000). Mating with a sister should simply add offspring to a male's reproductive output, without forfeiting other mating opportunities. Females should also benefit: their direct fitness is not depressed by inbreeding (present study) nor by mating with a polygynous male (Bouteiller and Perrin 2000). Thus, enhancing the reproductive output of related males increases female inclusive fitness (Parker 1979, 1983; Smith 1979; Waser et al. 1986; Perrin and Mazalov 2000). In this context, females should seek related partners only, rather than random partners within their neighborhood, as observed.

One possible explanation is that shrews lack the basic cognitive abilities necessary for familiarity-based recognition. This would also explain why common shrews (*Sorex araneus*) have to rely on multiple mating to limit the costs of inbreed-

ing depression (found to be high in this species; Stockley et al. 1993), rather than on avoidance of related partners.

Alternatively, it might be that choice is costly. Females are likely under strong selection to rear as many litters as possible within their single breeding season. Fertilization must occur immediately after parturition, if costly delays are to be avoided. Similarly, young females from first litters are fertilized as soon as weaned, because the time available before the season ends is extremely limited. Females may thus be under strong selective pressure to accept the first available partner they meet (for a similar argument, see Keller and Arcese 1998).

Finally, the marked breeding synchrony that necessary results from these time constraints presumably limits the number of partners that a male may fertilize (Say et al. 2001). As a result, choosing a sister would jeopardize other mating opportunities, thereby suppressing the potential benefits of inbreeding. In such a case, females have no special reason to focus on related partners, and matings should be random within local neighborhoods.

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#### APPENDIX

The relatedness,  $r_i$  among the two mating partners of a focal pair  $i$  can be obtained as (Hamilton 1971):

$$r_i = \frac{2F_{ST_i}}{1 + F_{IT}} \quad (A1)$$

where  $F_{ST_i} = (\theta_i - \alpha)/(1 - \alpha)$  measures their relative coancestry and  $F_{IT} = (F - \alpha)/(1 - \alpha)$  is the relative inbreeding in the population (excess of homozygotes). If assignments are correct, then the observed coancestry,  $\theta_i$ , among the putative mating partners (probability of identity of two alleles randomly sampled from each) should equal the homozygosity of their putative offspring:  $\theta_i = 1 - H_i$ , where  $H_i$  is offspring heterozygosity). Similarly, the average coancestry in the population is given by  $\alpha = 1 - H_T$  (where  $H_T$  is the expected heterozygosity) and the average fixation index (probability that the two alleles from the same individual are identical) is given by  $F = 1 - H_o$  (where  $H_o$  is the observed heterozygosity). Substituting these expressions in (A1) provides the expected relation between the observed heterozygosity of focal offspring with the relatedness among their putative parents:

$$H_i = H_T - r_i \left( H_T - \frac{H_o}{2} \right). \quad (A2)$$

Thus, these offspring should have a heterozygosity equal to Hardy-Weinberg expectation if their putative parents have average relatedness ( $r_i = 0$ ) and equal to half the observed heterozygosity in case of maximal relatedness among their putative parents ( $r_i = 1$ , which corresponds to selfing). The relation is linear in between.