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THE TRANSITION TO SOCIAL INBRED MATING SYSTEMS IN SPIDERS: ROLE OF INBREEDING TOLERANCE IN A SUBSOCIAL PREDECESSOR

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Abstract.—The social spiders are unusual among cooperatively breeding animals in being highly inbred. In contrast, most other social organisms are outbred owing to inbreeding avoidance mechanisms. The social spiders appear to originate from solitary subsocial ancestors, implying a transition from outbreeding to inbreeding mating systems. Such a transition may be constrained by inbreeding avoidance tactics or fitness loss due to inbreeding depression. We examined whether the mating system of a subsocial spider, in a genus with three social congeners, is likely to facilitate or hinder the transition to inbreeding social systems. Populations of subsocial *Stegodyphus lineatus* are substructured and spiders occur in patches, which may consist of kin groups. We investigated whether male mating dispersal prevents matings within kin groups in natural populations. Approximately half of the marked males that were recovered made short moves (< 5m) and mated within their natal patch. This potential for inbreeding was counterbalanced by a relatively high proportion of immigrant males. In mating experiments, we tested whether inbreeding actually results in lower offspring fitness. Two levels of inbreeding were tested: full sibling versus non-sib matings and matings of individuals within and between naturally occurring patches of spiders. Neither full siblings nor patch mates were discriminated against as mates. Sibling matings had no effect on direct fitness traits such as fecundity, hatching success, time to hatching and survival of the offspring, but negatively affected offspring growth rates and adult body size of both males and females. Neither direct nor indirect fitness measures differed significantly between within patch and between-patch pairs. We tested the relatedness between patch mates and nonpatch mates using DNA fingerprinting (TE-AFLP). Kinship explained 30% of the genetic variation among patches, confirming that patches are often composed of kin. Overall, we found limited male dispersal, lack of kin discrimination, and tolerance to low levels of inbreeding. These results suggest a history of inbreeding which may reduce the frequency of deleterious recessive alleles in the population and promote the evolution of inbreeding tolerance. It is likely that the lack of inbreeding avoidance in subsocial predecessors has facilitated the transition to regular inbreeding social systems.

Key words.—DNA fingerprinting, inbreeding avoidance, inbreeding tolerance, inbreeding rate, kin-structured population, life-history traits, sibling mating; sex-specific dispersal.

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One of the major forces shaping the evolution of mating systems is selection to avoid mating with close relatives. There are two hypotheses that may explain the potential costs of inbreeding (Charlesworth and Charlesworth 1987). The partial dominance hypothesis states that inbreeding depression stems from the increase in frequency of homozygous deleterious recessive alleles, some of which may be purged through continued periods of inbreeding. According to the overdominance hypothesis, heterozygotes have superior fitness so that inbreeding, leading to homozygosity, causes a fitness decline. According to this hypothesis, periods of inbreeding do not reduce costs through purging if they are associated with lower fitness of both homozygotes relative to the heterozygote.

Animals employ a variety of mechanisms to avoid inbreeding. Prior to mating, natal dispersal, sex-specific mating dispersal, or incest taboos reduce the risk of mating with relatives (Pusey and Wolf 1996, Perrin and Goudet 2001). Behaviors such as polyandry may reduce the likelihood that a female will utilize incompatible sperm or sperm from closely related males, either directly or through cryptic post-mating mechanisms (Zeh and Zeh 1996; Tregenza and Wedell 2002; Stockley 2003). Yet, inbreeding avoidance may be constrained by costs such as high mortality rates during dispersal or missed mating opportunities. Inbreeding mating systems can therefore be selected when the costs of avoidance are greater than the costs of inbreeding depression (Waser et al. 1986). The balance between tolerance and avoidance of inbreeding is influenced by multiple factors, for example the prevalence of recessive deleterious alleles in a population (Charlesworth and Charlesworth 1987); habitat stability and risks associated with dispersal and searching for nonconsanguineous mates (Hölldobler and Wilson 1990; Perrin and Goudet 2001; Yoder et al. 2004); the proximity and relatedness of potential mates and effective population size (Shields 1993; Lande and Barrowclough 1987);

The balance between inbreeding tolerance and avoidance is particularly interesting in the case of social organisms. In cooperatively breeding animals, reproductive siblings interact in close proximity, and most social species have evolved mechanisms to prevent or reduce inbreeding (Emlen 1991; Cook and Crozier 1995; Pusey and Wolf 1996; Cockburn 1998). In contrast, high levels of inbreeding are typical of the permanently social spiders (Riechert and Roeloffs 1993; Avilés 1997). In the social spiders, one or a few mated females typically initiate new colonies, and offspring mature and reproduce within the natal colony. There is little or no movement of spiders among colonies, and such groups may persist for several generations. Primary and adult sex ratios are female biased and the species that have been investigated show low levels of allozyme variation compared to nonsocial spiders, suggesting that regular inbreeding is the rule rather than the exception (Lubin and Crozier 1985; Smith 1987; Roeloffs and Riechert 1988; Riechert and Roeloffs 1993; Smith and Hagen 1996; Aviles 1997; Evans and Goodisman 2002).

Social behavior is rare but phylogenetically widespread among spiders; 20 known cooperative species represent at least 12 independent origins of sociality (review in Avilés 1997, D'Andrea 1987). With the exception of *Mallos*, sociality has evolved in families where subsocial behavior (maternal care) is widespread (Buskirk 1981; D'Andrea 1987; Avilés 1997, Bond and Opell 1997). Subsocial spiders live a solitary life as adults and extended maternal care results in the formation of temporary cooperative groups consisting of a female and her offspring. Subsocial spiders are sometimes referred to as periodic-social whereas the social spiders are permanently group living (Avilés 1997). Regularly inbreeding social groups appear to originate from solitary subsocial ancestors through prolonged associations of juveniles, suppression of pre-mating dispersal and mating among siblings (Buskirk 1981; Avilés 1997). Because the majority of spider species are outbreeding, this hypothesis implies a transition from predominantly outbreeding to inbreeding mating systems in conjunction with the evolution of sociality.

Hence, the role of inbreeding tolerance is a major issue in the transition to permanent sociality. Is inbreeding tolerance a prerequisite or a constraint for the evolution of inbred social systems? The answer can be sought within the subsocial ancestors. Three factors are important: benefits of cooperative social behavior, costs of inbreeding, and costs of search for unrelated mates. If inbreeding incurs fitness costs, the transition to inbred social systems would be constrained by selection for inbreeding avoidance, and major benefits of group living would be necessary to outweigh the initial inbreeding costs. However, a history of inbreeding within the subsocial predecessors would lead to a reduction in the frequency of recessive deleterious alleles (Barrett and Charlesworth 1991; Crnokrak and Barrett. 2002), reducing the initial cost of transition to regular inbreeding. In addition, inbreeding might be tolerated if the cost of alternatives, such as long distance mating dispersal, were greater than the cost of inbreeding depression. Therefore, the investigation of the mating system of subsocial species that are closely related to permanent-social species is likely to cast light on the role of inbreeding tolerance or avoidance in the evolution of regularly inbreeding social systems.

Generally we assume subsocial spiders to have an outbred mating system, based on the presence of premating dispersal of one or both sexes (Schneider 1997; Avilés and Gelsey 1998; Powers and Avilés 2003), and adult sex ratios close to 1:1 (Lubin 1991). Yet, there are limited genetic data to substantiate this assumption. Several studies report genetic differentiation within and among populations of subsocial spiders, as a result of philopatry, restricted dispersal, or founder events (Johannesen et al 1998, Johannesen and Lubin 1999, 2001; Bond et al 2001; Pedersen and Loeschcke 2001). In contrast, population genetic studies of solitary species document low genetic differentiation despite a bias towards investigations of species that are suspected to be restricted in either dispersal ability or habitat availability (Ramirez and Fandino 1996; Boulton et al 1998; Ramirez and Haakonsen 1999; Tso et al 2002; Bonte et al 2003).

We present a study of the mating system and inbreeding tolerance in the subsocial spider *Stegodyphus lineatus* (Eresidae). The genus *Stegodyphus* contains three independently derived permanent-social species that fall into three species groups (Kraus and Kraus 1988). A current reconstruction of the phylogenetic tree using molecular data appears to confirm this structure (Jes Johannesen, pers. comm.). The *Stegodyphus* phylogeny shows repeated origins of social inbred mating systems, suggesting a central role of inbreeding tolerance in subsocial ancestors in the transition to permanent sociality. In *S. lineatus*, we investigated (1) the likelihood of mating with relatives, (2) the presence of inbreeding avoidance mechanisms, and (3) the degree of tolerance of inbreeding. Populations of the *S. lineatus* are often patchily distributed, and isolated clusters of spiders can consist of sibling groups established by the offspring of a single female (Johannesen and Lubin 1999; 2001). Such a population structure suggests a potential for mating among close kin. If mating with relatives results in inbreeding depression, inbreeding avoidance mechanisms such as sex-specific mating dispersal or discrimination of kin as mates should evolve (Motro 1991; Perrin and Goudet 2001).

We investigated male dispersal patterns during the reproductive season in geographically separated populations of *S. lineatus*, asking whether males mate locally within the patch in which they mature, or if they engage in long-range dispersal that will take them to unrelated females in more distant patches. In mating experiments, we investigated whether *S. lineatus* discriminate against kin as mates. Bilde and Lubin (2001) found reduced aggression among sib-groups of juvenile *S. lineatus*, showing the ability to discriminate kin from nonkin. We presented females with either a related or an unrelated male comparing mating success and behavior between inbred and outbred crosses. Subsequently we quantified fecundity and life-history traits of the offspring to determine the effect of inbreeding on fitness. Fecundity, hatching success, and offspring survival are traits that are directly related to fitness and were expected to show strong inbreeding depression, whereas offspring body size is more indirectly related to fitness and should be less affected by inbreeding (Mousseau and Roff 1987; DeRose and Roff 1999).

Inbreeding depression is less severe under low rates of inbreeding (Day et al. 2003), hence the degree and rate of inbreeding influences the magnitude of fitness loss. We therefore determined the effects of two levels of inbreeding in *S. lineatus*: (1) in crosses between full siblings, to maximize the inbreeding coefficient (full-sib mating experiment) and (2) in crosses within naturally isolated patches of spiders in the field, to capture the natural range of inbreeding levels in field populations (patch-mating experiment). Finally, we determined the relatedness of mated pairs in the patch-mating experiment with DNA-fingerprinting to test the assumption that within-patch mates are more closely related than between-patch mates.

Materials and Methods

Natural History

Stegodyphus lineatus is a web-building spider with a circum-Mediterranean distribution. The spiders occupy thick, permanent silk nests and they rarely relocate (Ward and Lubin 1993). The spiders are annual and semelparous with extensive maternal care. The mating season starts in March, when the first males mature, and egg-laying occurs from April to June. Mature males abandon their nests in search of mates and they often cohabit with adult females for several days (Schneider and Lubin 1998). Females are sedentary and may be visited by multiple males during the mating season (Schneider and Lubin 1996). The female releases the young (40–140 spiderlings) from the egg sac and feeds them actively by regurgitation for approximately two weeks, before she is finally consumed by the young (Kullmann 1972). After matriphagy, the offspring remain as a family group in the maternal nest for a variable length of time, ranging from two weeks to three months (D'Andrea 1987).

Dispersal

We marked all *S. lineatus* nests in two populations in Greece and three populations in Israel. All spider nests in the sample populations were marked individually before the onset of the mating season, defined by the date when we found the first male cohabiting with a female. Males were individually marked with up to four watercolour dots on the dorsal side of the abdomen after they matured in their nests (resident males) or if they were found cohabiting with females in marked nests (immigrant males). We assumed that all resident males in the population were marked in their own nests; therefore unmarked males that we found with females were classified as immigrants. Each nest was inspected daily for the presence of males until the end of the mating season when no more males were found.

In February 1991, nests of *S. lineatus* inhabited by immature spiders were marked at two sites on the Greek island of Karpathos (27°08′E; 35°25′N). Neither site had populations defined by distinct borders. Site 1, Afiartis, consisted of two parallel rows of *Pistacia lentiscus* hedges (about 500 m long and 1.5 m high) that were close to the southeastern shore of the island. Site 2, Arkasa, was a slope on the southwest coast and characterized by low vegetation (<0.5 m). In Site 1, the spiders were confined to the hedges, but there were more hedges to the north and south of the study site. Site 2 was a more or less evenly populated slope where only a subsample could be studied. We marked all spiders along a transect 2-m wide and 1-km long running roughly in an east-west direction. The first adult males appeared in the first week of April and the last males were marked six weeks later.

In February 1995 we marked all nests of immature *S. lineatus* in a wadi (dry wash) on the Avdat plateau (34°46′E; 30°48′N) in the Negev Desert, Israel (Avdat I). The first males appeared in the middle of March and the last males were seen in the middle of May. In February 2002, we marked all *S. lineatus* nests in a wadi in the upper Zin watershed (34°42′E; 30°49′N) in the Negev Desert. Males were found from late March to the end of May. In March–May 2003, we recorded male movement patterns and measured distances moved by marked resident males, but not immigrant males, to females in a third Negev population on the Avdat plateau (Avdat II). The three Negev sites were separated from one another by 1.5–2 km.

In the Negev populations, *S. lineatus* spiders were aggregated in patches. Several spider nests could occur either on one shared shrub or on a cluster of shrubs, and we defined such a cluster of spider nests as a patch. Patches were considered discrete when the distance to the nearest neighboring spiders was at least six meters, which was the maximum-dispersal distance recorded for juveniles (Lubin et al. 1998). Allozyme data indicate that spiders within a patch may consist of siblings (Johannesen and Lubin 1999, 2001). We determined whether males mated within or between such patches. The two Karpathos populations had continuous rather than a patchy distribution and here the distance from a male nest to nearest females were measured to determine whether males moved to the nearest or to more distant females.

Males move at night, and during the day they remain in the nests of females. Males that cohabit with mature females usually succeed in mating (Schneider and Lubin 1996; Maklakov and Lubin 2004). Therefore, the presence of a male in the nest of a mature female was considered a mating event.

Mating Experiments

All spiders used in mating experiments were collected as subadults from natural habitats in Israel (Negev and Judean Deserts) and brought to the Sede Boqer Campus of Ben Gurion University, located in the Negev Desert. Spiders were kept individually in plastic containers with mesh lids (height 10 cm, diameter 5 cm) and reared to maturity on a diet of desert locusts (*Locusta migratoria*), crickets (*Acheta domestica*), and beetles (*Tenebrio molitor*). Offspring of singly mated females were raised for full-sib mating experiments (see below) under similar conditions. We kept the spiders outdoors under ambient conditions, partially protected against direct sun by shade cloth. Subadult and adult females were transferred to an outdoor spider house consisting of a greenhouse frame covered with fine wire mesh, which provided natural weather conditions and photoperiod. Nests of females were attached to a chicken wire fence inside the spider house, and each nest and web was covered with a mesh box (height = 12.5 cm, length = 12.5 cm, depth = 10 cm) to prevent the spiders from wandering. The spiders constructed capture webs inside the mesh boxes. We performed mating experiments in the spider house by placing males at the edge of females' webs. Prosoma width of all spiders was measured after the final molt.

Mating Behavior

We tested whether spiders possess behavioral avoidance mechanisms that reduce the likelihood of mating with close kin (1) by pairing females with full sibling or nonsib mates, and (2) by pairing females with a male from her own patch or a different patch. We performed mating experiments by placing a male at the edge of the web of a female. When the male encountered the female's web he immediately initiated searching behavior that led him to the entrance of the nest. During this phase the male might vibrate the web in bouts of one to five vibrations before he entered the nest (Maklakov et al. 2003). In most cases, copulation would occur within minutes if the female was receptive. Otherwise, the male would enter the nest and sit in the entrance in a ''face out'' position. In these cases, mating occurred later (Schneider and Lubin 1996; Maklakov and Lubin 2004). Females normally remained passive inside their nest during courtship and then came forward to the nest entrance to mate. Occasionally they reacted aggressively towards males. We observed the spiders until mating occurred or until the male occupied the entrance to the females nest. Then we closed the cage and left the male with the female for five days, subsequently removing the male and leaving the female to produce an egg sac.

In a few cases the male did not court the female or the female reacted aggressively towards the male and chased him away. In such cases, we recorded male and female behavior until the male was chased away or until he settled motionless at the edge of the web (observations lasted up to two hours). We then removed the male and regarded the trial as a rejection. The female was later paired with another mate in the same type of mating trial (e.g., sib or non-sib). In a few cases the male would not initiate searching behavior and was removed either if he left the female web or when he had stayed motionless for two hours; these cases were too few to analyse statistically. We recorded the following variables during mating trials: mating success (yes/no), female aggressiveness (present/absent); occurrence (present/absent) and number of courtship vibrations; lag time for males to enter the female's nest (in 2000 and 2001); time to copulation; and copulation duration (2001 only). Categorical dependent variables (mating success, female aggression, male vibration) were analyzed with logistic regression using relatedness as the independent variable and female and male size (prosoma width) as covariates. Continuous dependent variables (time for males to enter the female's nest, time until mating and mating duration) were analyzed with analysis of covariance with relatedness as the independent variable and female and male size as covariates. Continuous variables were log (x + 1) transformed when necessary. Behavioral data from sib/non-sib mating experiments obtained in the two consecutive years (2000 and 2001) were compared with logistic regressions (categorical variables) or two-way ANOVA (continuous variables) with relatedness and year as independent variables. Statistical analyses were performed with Systat 10 and JMP 5 (Statistical Analysis System Institute).

Full-Sibling–Nonsibling Mating

Full siblings for mating experiments were the offspring of females mated to one randomly selected virgin male. The offspring were then raised to adulthood for full-sib mating experiments. Ten to 20 spiderlings from each family were raised individually following the procedure described above. The mating experiments were performed the following year, when the offspring matured. Females were presented either with a sib male or a male from a different family. We recorded mating behavior as described above and tested for differences in behavioral components between sib matings and nonsib matings. We recorded eggsac production, hatching time (from eggsac production), clutch size, and percent of eggs hatched. Samples of F_1 offspring from the inbred and outbred crosses (10–15 individuals from each family) were raised following the standard procedure, and we collected data on average juvenile mass at dispersal, days to dispersal (from hatching), number of dispersing young, survival to adulthood, growth rate, development time, and adult body size of F_1 offspring. Growth rate (mg/day) was determined over two months of development.

We controlled for the possible influence of body size by including spider prosoma width as a covariate in all statistical analyses. Female fitness variables (fecundity, hatching success, juvenile body mass) were analyzed with analysis of covariance (ANCOVA). All models had the breeding treatment (full-sib/nonsib or within patch/between patch) as the main effect and female size (measured as prosoma width in mm) as covariate. Analysis of hatching time of eggsacs included egg-laying date (Julian day) as covariate; ANCOVA of average juvenile mass (mg), and days to dispersal included the number of young as covariate; in analyzing the number of dispersing offspring, hatching date was a covariate. The effect of breeding treatment on offspring growth rate (mg/day), developmental time (days), survival to adulthood, sex of adult spiders, and adult body size (prosoma width in mm) was analyzed with nested ANOVA where data on siblings were nested within family (mother). Development time was included as covariate in the analysis of body size.

Male developmental time was bimodal in both the full-sib mating and the patch mating experiment. In the full-sib mating experiment 20 male offspring had a prolonged and non-normally distributed maturation time (median: 462.5 days, range 384–494 days) and were therefore excluded from the statistical analysis; this did not affect the results qualitatively. In the patch mating experiment males were separated for the analysis into two groups: one with a developmental time less than 375 days and another in which males matured in the interval between 375 and 550 days. A few females with a developmental time longer than 550 days were excluded from the analysis.

We performed the full-sib mating experiments in two successive years, 2000 and 2001. In 2000, when the experimental procedure was first established, females matured late in the season and relatively few of the mated females produced eggsacs. Therefore, only a subset of data is presented from the 2000 experiment.

Males from the mating experiments were preserved for relatedness analysis (see below) where genetic distance among full siblings was assessed with DNA fingerprinting.

Within and between Patch Mating

In the Negev Desert, populations of *S. lineatus* are often scattered in distribution with a mixture of single individuals and patches with multiple individuals. A patch consists of one or a few adjacent shrubs containing several spiders. Previous allozyme studies showed higher average relatedness within patches than between patches in the same population (Johannesen and Lubin 1999, 2001). We performed an experiment to test whether there are mechanisms to prevent mating with patch mates and to examine fitness consequences of within-patch matings. The experiment was designed to duplicate the actual degree of relatedness between spiders in the field, which we expect to range in a continuum from full sibs and half sibs to unrelated individuals.

In late summer and autumn of 2000, we identified isolated patches of dispersed *S. lineatus* young in natural habitats in the Negev Desert in the Avdat Plateau area. Isolated patches contained several spider nests sharing a shrub or a cluster of shrubs and were separated by at least 10 meters in all directions from the nearest neighboring spider. We marked the locations of the shrubs and the location of all individual spiders in the patch but left the spiders otherwise undisturbed. In the following year, (March 2001), we collected the subadult spiders and raised them to adulthood as previously described. When females matured, they were presented either with a male from their own patch or with a male from a distant patch that was more than 100 meters away. We recorded mating behavior and took data on reproductive values and fitness traits for the F_1 generation as described in the full-sib experiment to compare within-patch and between-patch crosses.

Following mating trials, we collected the males and preserved them in 80% ethanol for DNA extraction (below). Approximately 10 days after the young had hatched and prior to matriphagy, we removed one leg IV from each female for DNA extraction. We determined relatedness between patch mates and nonpatch mates by use of genetic fingerprints (TE-AFLP; Vos et al., 1995, Van der Wurff et al. 2000).

Relatedness

DNA fingerprinting

Tissue samples for DNA were preserved in 80% ethanol and stored at 4° or −20° C and extracted using DNEasy tissue preparation kits (Qiagen). Genetic variation among spiders was assayed using TE-AFLP banding patterns (three enzyme amplified fragment length polymorphisms; Vos et al. 1995; Van der Wurff et al. 2000). DNA digestion and ligation to adaptors are described in Van der Wurff et al. (2000). A subset of the DNA fragments produced by the digestion/ligation reaction was selectively amplified using primers ‘‘XbaI-CC’’ and ‘‘BamHI-C’’ as described by Van der Wurff et al. (2000) and Palmer et al. (2003). Presence or absence of AFLP bands up to approximately 200 bp in length was recorded manually.

Band analysis

Each spider was characterized by a DNA ‘‘fingerprint’’ or set of AFLP bands. Pairwise distance measures among unique banding patterns were estimated using the method of Excoffier et al. (1992) as implemented in the program RAPDistances (Armstrong et al. 1994). Using this metric, the distance between individuals i and $j = (n)(1 - [n_{ij}/n])$, where n is the total number of bands scored, and n_{ij} is the number of bands present in both individuals. The pairwise distance matrix was used in an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to estimate Φ_{ST} among patches and among sib groups. Φ_{ST}, which can take values from 0 to 1, is analogous to F_{ST} for codominant markers and indicates the proportion of observed genetic variation that is explained by differentiation among populations. Here we analyzed the proportion of observed genetic variation that could be attributed to genetic differentiation among patches.

Sixty four spiders from 24 patches were successfully fingerprinted (one to five individuals per patch). The 20 patches that provided two or more fingerprinted individuals were used in an AMOVA analysis to estimate genetic differentiation among patches. A similar AMOVA analysis was performed on 16 groups of two to eight full siblings (45 spiders) to determine the extent of genetic differentiation among sets of full siblings. To test for significance, 2000 permutations were performed comparing observed population subdivision to that obtained when individuals were assigned at random to 20 groups of the same sizes as the observed patches (for differentiation among patches) or 16 groups of the same sizes as the observed sibling groups (for differentiation among sibships).

In the mating experiments, males and females for pairs were drawn either from the same patch (patch mates) or from different patches (nonpatch mates). We compared mean genetic distance between mates from the same patch, mates from different patches, and pairs of sibships. We also used AMOVA permutation to compare genetic similarity of 16 patch mate pairs to 2000 permutations of 16 pairs drawn at random from the same set of 32 spiders, and genetic similarity of 11 nonpatch mate pairs to 2000 permutations of 11 pairs drawn at random from the same set of 22 spiders.

RESULTS

Dispersal

Thirty to sixty percent of male movements were five meter or less, which means that a large proportion of males mated in the patch where they matured (Fig. 1). In Karpathos, nine

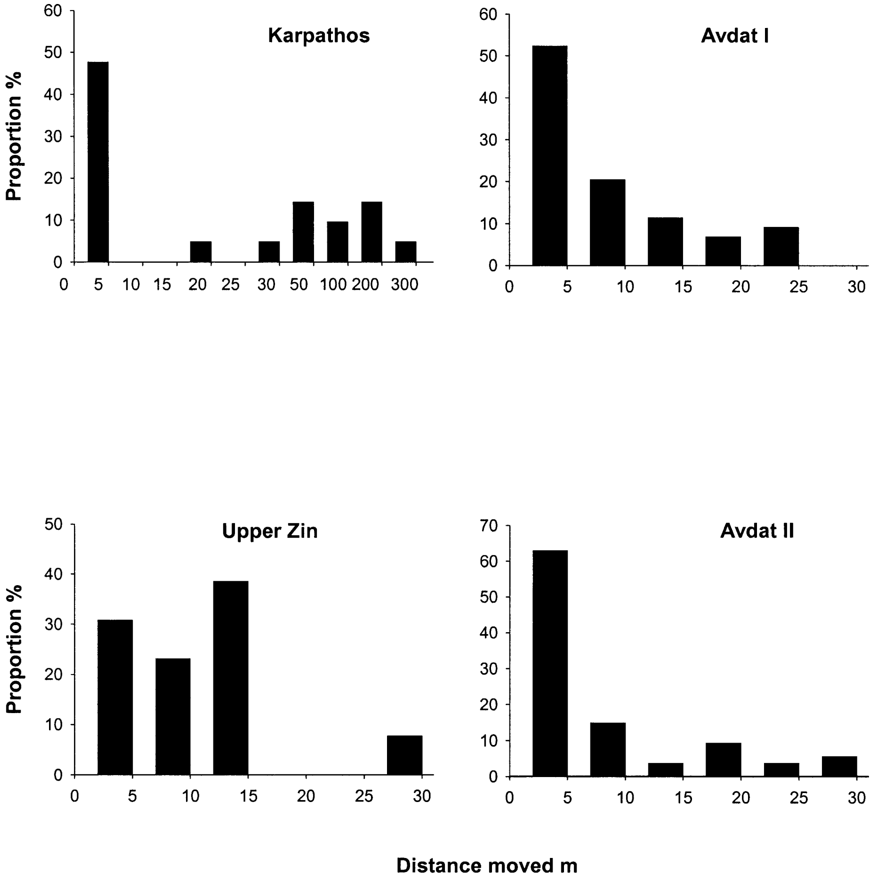

FIG. 1. Frequency distribution of distances moved (m) by *Stegodyphus lineatus* males in five populations: two locations on Karpathos Island (Greece) (data combined for Afiartis and Arkasa, $n = 21$) and three localities in the Negev Desert, Avdat I ($n = 44$), Avdat II ($n = 54$), and upper Zin wadi ($n = 13$).

moves were to the female nearest to the male at a median distance of 1.39 m (lower and upper quartiles: 0.27 and 8.69 m, range: 0.19–35.5, $n = 21$ moves, both populations combined). The median distance moved by these nine males was not significantly different from the median distance between all resident males and their nearest females (median: 1.66 m, lower and upper quartiles: 0.9 and 6.37 m, range: 0.18–44 m, $n = 20$; Mann-Whitney $U = 106.5$, $P = 0.4$). The remaining 12 moves were significantly longer than the distance to the nearest female (median: 62.1 m, lower and upper quartiles: 9.84 and 103.54 m, range 2–242.89 m; $U = 100$, $P = 0.001$, $n = 21$). In the Negev populations, median distances moved were 4–8 m and the longest moves were less than 30 m (Table 1).

In the two Karpathos populations, recovery of resident males was low whereas immigrant males constituted 55–70% of the males found. In comparison, the proportion of immigrant males found in the Negev populations was low (Table 1). The difference can be explained by the nature of the two study sites on Karpathos. In contrast to the Negev, the Karpathos sites were not isolated patches and males that matured in nests at the edges of the sites had a lower probability of recapture. Hence, relative to the locally confined populations in the Negev, recapture rates were expected to be lower and immigration was expected to be higher in Karpathos.

In all populations, males were observed in nests of more than a single female. In Karpathos, eight males (42%) mated with more than one female. In Avdat I, 11 males (30%) visited more than one female; in Upper Zin, four (31%); and in Avdat II, 13 (46%). In all three Negev Desert populations, males that mated with more than one female moved slightly but significantly shorter distances to their first female than to subsequent females: Avdat I: 1st move 6.61 m + SE 1.69, 2nd move 8.29 + SE 1.38; Avdat II: 1st move 6.75 m + SE 1.50, 2nd move 7.52 + SE 1.46; Upper Zin: 1st move 6.04 m + SE 1.48, 2nd move 16.66 + SE 3.95; (two-way ANOVA, log-transformed distances, with population and number of females visited, first or subsequent, as factors, $F_{1,105} = 6.099$, $P = 0.015$, $n = 111$). Neither population nor the interaction between population and female number was significant. A similar result was obtained when we compared male moves to their first and to their second females only, rather than to all subsequent females ($F_{1,73} = 5.85$, $P = 0.018$, $n = 79$).

Using data from two sampling populations, Avdat I and II, 33% of the females mated with more than one male (females were visited by up to six males; $n = 67$).

Full-Sibling–Nonsibling Mating

The degree of relatedness as experienced in full-sibling versus nonsibling matings had no significant effect on mating

TABLE 1. Demographic and dispersal data from males of five populations: the proportion of resident and immigrant males recovered in nests of females (the total number of marked males from which the given proportion were recovered is given in parentheses). The median distance moved is given in m (*n*, range).

Location		% Resident males recovered	% Immigrant males recovered	Median distance traveled
Karpathos	Afiartis	38 (24)	55 (29)	2 (13, 0.18–104)
	Arkasa	29 (28)	70 (64)	62.1 (8, 15.2–242)
Negev Desert	Avdat I	74 (38)	39 (24)	4.75 (44, 0.5–25)
	Upper Zin	23 (40)	18 (9)	8.7 (13, 0.2–28.3)
	Avdat II	61 (46)	—	4.36 (54, 0.25–29.1)

success, female aggression or various components of male courtship (Table 2). There was no difference between years in female aggression (logistic regression: $-\log$ likelihood = 16.148, df = 2, $P = 0.413$; relatedness: $\chi^2 = 0.05$, $P = 0.817$; year: $\chi^2 = 1.71$, $P = 0.194$, $n = 58$) nor in the time from initiation of courtship until the male entered the female's nest (two-way ANOVA, (time to enter nest log transformed), $F_{2,46} = 1.0586$, $P = 0.355$; relatedness: $F = 1.321$, $P = 0.256$; year, $F = 0.715$, $P = 0.401$). However, the proportion of vibrating males differed between years (logistic regression: $-\log$ likelihood = 30.307, df = 2, $P = 0.075$; relatedness: $\chi^2 = 0.32$, $P = 0.573$, year: $\chi^2 = 4.42$, $P = 0.035$, $n = 48$; Table 2).

We found no difference between inbred and outbred crosses in hatching time of eggsacs, total clutch size, and the percent of eggs that hatched (Table 3). Average juvenile body mass of inbred spiderlings was lower than that of outbred offspring also when accounting for differences in body mass (covariate) caused by the variable number of young in the clutch (Table 3). The time to disperse and the number of dispersing spiderlings did not differ between treatments.

Inbred offspring had a lower growth rate than outbred offspring (Fig. 2A, Table 4). Growth rate was strongly influenced by family origin, whereas the sex of the young had no effect. Total development time was longer for females than for males and the sexes were therefore analyzed separately. Development time was longer for inbred than for outbred spiders, particularly for males (Fig. 2B, Table 4), and there was a strong family effect on development time for both sexes. Breeding treatment did not influence survival to adulthood, which was 84% of inbred offspring ($n = 163$) and 81% for outbred offspring ($n = 112$, Table 4). We found no difference in sex among adult offspring (inbred offspring: 72 females and 61 males, outbred offspring: 50 females and 45 males; $\chi^2 = 0.001$, $P = 0.997$). Adult body size of inbred offspring was lower than that of outbred offspring (Table 4, Fig. 2C). A similar result was found in the 2000–2001 experiment (Table 4, Fig. 2D), in which inbred adult offspring were smaller than outbred offspring. However, the effect, was stronger for males than for females.

The decline in the value of fitness traits, calculated as inbreeding depression coefficients, was less than 10% for most traits, with higher values for juvenile body mass (21%) and growth rate (38%).

Within and between Patch Mating

We found no difference between patch mate pairs and nonpatch mate pairs in mating success, female aggression, and occurrence of male courtship vibrations (Table 5), or in the number of male courtship vibrations, the time for males to enter the nest, the time until mating occurred and the duration of the first mating (Table 5).

Time to hatching, clutch size, and percent eggs hatched did not differ when mates were drawn from within the same patch or from different patches (Table 6). Average mass of juveniles from between patch matings was lower than that of offspring from within patch matings; however, this difference was not explained by breeding treatment but rather by a strong covariance with the number of young in the clutch (Table 6). Breeding treatment did not influence the average time to dispersal whereas the number of dispersing young

TABLE 2. The degree of inbreeding (sib/nonsib) mating and its effect on mating and courtship in two years. None of the variables differ significantly. Data were log(x + 1) transformed before being used in ANCOVA with female and male size as covariates. For continuous data, median and range are given.

	Year	Full-sib mate	*n*	Unrelated mate	*n*	Test statistic
Mating pairs	2001	18	23	11	16	*$\text{Log-l}_3 = 21.679$
	2000	10	12	9	9	$\text{Log-l}_3 = 6.604$
Aggressive females	2001	1	23	1	17	$\text{Log-l}_3 = 6.554$
	2000	2	10	1	9	$\text{Log-l}_3 = 36.567$
Vibrating males	2001	14	18	6	11	$\text{Log-l}_3 = 14.441$
	2000	3	10	4	9	$\text{Log-l}_3 = 11.192$
Vibration number	2001	3.5 (0–17)	18	1 (0–22)	11	$U = 78.5$
Time to enter nest (sec)	2001	100 (2–2893)	17	81 (2–4800)	13	$F_{3,26} = 0.394$
	2000	197.5 (0–1087)	10	17 (0–1880)	9	$F_{3,15} = 0.248$
Time to mate (sec)	2001	313 (90–5439)	15	955 (238–3297)	7	$F_{3,19} = 2.153$
1st mating duration (sec)	2001	1222.5 (415–4262)	16	1361 (524–4037)	7	$F_{3,19} = 0.551$

* Log-l stands for the log-likelihood ratio derived from a logistic regression with female and male sizes as covariates.

TABLE 3. ANCOVA of the effect of breeding treatment on fitness variables (mean and SE) of offspring resulting from crosses between full-sibs (inbred, $n = 15$) or nonsibs (outbred, $n = 8$). Average juvenile mass is measured as total mass of young/number of young. Statistical results are given for the full model and when significant also for the factor and covariates.

Source	Inbred	Outbred	Test statistics
Clutch size	68.78 (5.03)	74.33 (5.23)	$F_{3,17} = 0.469$
Hatching time (days)[3]	25.64 (0.41)	24.0 (1.53)	$F_{3,22} = 0.9$
Hatching percent[1]	88.3 (0.028)	85.9 (0.06)	$F_{3,19} = 1.029$
Average juvenile mass (mg)[2]	5.32 (0.25)	6.74 (0.59)	$F_{3,19} = 9.61$**
Breeding treatment			$F_{3,19} = 14.591$*
Female size			$F_{3,19} = 0.312$
No. of young in clutch			$F_{3,19} = 20.119$**
Time to disperse (days)[4]	41.06 (1.21)	42.9 (1.84)	$F_{3,18} = 2.636$
No. of dispersing young[5]	60.57 (4.72)	65 (6.76)	$F_{3,19} = 0.787$

* $p < 0.01$, ** $p < 0.001$.

[1] Arcsine and [2] square root transformed for analysis; female size (prosoma width) was taken as covariate for all analyses. Further covariates: [2] clutch size, [3] date of egg-laying, [4] clutch size, [5] hatching date.

was a highly significant covariate (Table 6), due to a strong negative relationship between number of young and time to dispersal ($r = 0.3$, $F_{1,54} = 23.451$, $P < 0.0001$). More spiderlings survived to dispersal in the between patch mating treatment, an effect that was not due to the breeding treatment but was related to covariance with the hatching date (Table 6).

Growth rate of offspring from within patch matings was higher than that of offspring from between patch matings, and growth rate was significantly affected by family origin (Fig. 3A, Table 7). We found no effect of breeding treatment on the total development time (Fig. 3B, Table 7). Development time for females was independent of family origin, whereas male development time was highly influenced by family origin, in particular those males with the shortest development time (< 375 days, Table 7).

Survival to adulthood was 64% for within patch mated offspring ($n = 311$) and 67% for between patch mated offspring ($n = 265$) and did not differ between breeding treatments (Table 7). There was no effect of breeding treatment

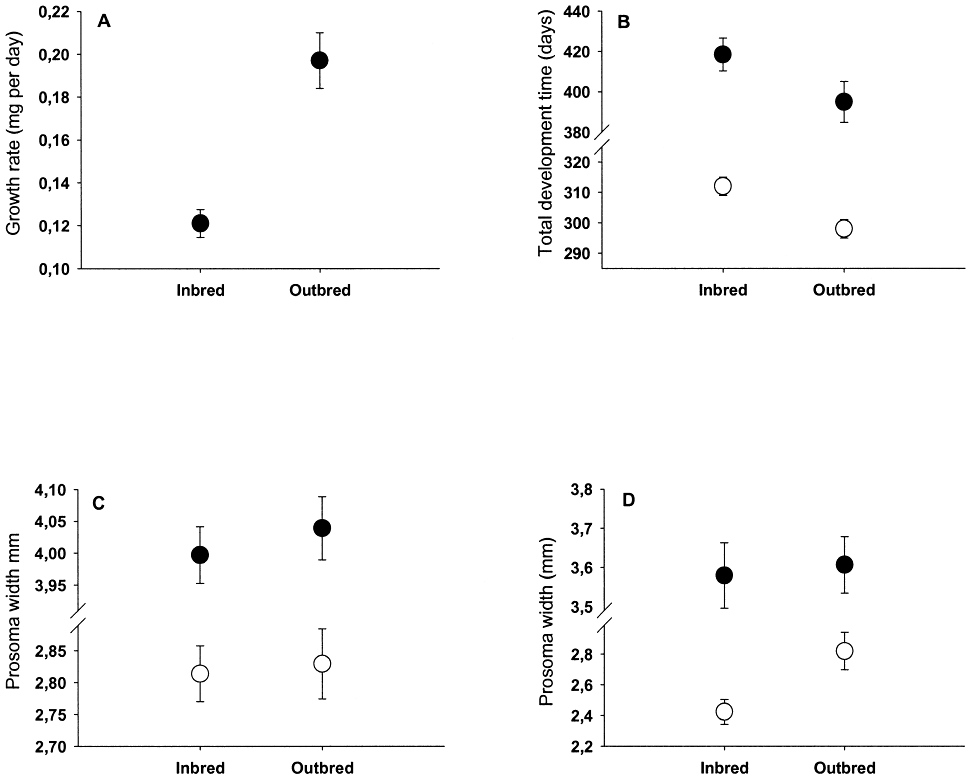

FIG. 2. Offspring life-history traits from inbred (sib mating) and outbred (nonsib mating) crosses of *Stegodyphus lineatus* spiders: (A) Offspring growth rate (mg per day) measured over a two-month period, 160 inbred and 114 outbred offspring; (B) total development time (days) from hatching to adult for 69 inbred and 50 outbred females (●, developmental time < 550 days), and 46 inbred and 49 outbred males (○, development time < 360 days); (C) adult body size (prosoma width in mm, 2001 experiment), 70 inbred and 50 outbred females (●), 49 inbred and 46 outbred males (○); (D) adult body size (prosoma width in mm, 2000 experiment), 16 inbred and 28 outbred females (●), 23 inbred and 20 outbred males (○).

TABLE 4. The effect of breeding treatment (sib/nonsib mating) on offspring fitness variables: (A) Offspring growth rate (mg per day, $n = 232$, square root transformed) measured over a two-month period; (B) total development time from hatching to adult (days), data for males (development time >360 days excluded, $n = 94$) were not transformed, data for females (development time >550 days excluded, $n = 119$) were log transformed; (C) probability of survival from hatching to adult ($n = 275$); (D) and (E) offspring adult body size (prosoma width in mm, log transformed) for 2001 (119 females, 94 males) and 2000 (44 females, 43 males), respectively. Nested ANCOVA was used to analyze growth rate, development time and, adult body size. Development time and adult sizes were analyzed separately for males and females, because these differed significantly for the two sexes. Probability of survival was analyzed by logistic regression. In all analyses, family was nested within breeding treatment.

		df	Test statistic
(A) Growth rate			
	Breeding treatment	1	$F = 33.414$***
	Family	25	$F = 3.932$***
	Sex	1	$F = 1.527$
(B) Development time			
Females	Breeding treatment	1	$F = 3.02$
	Family	25	$F = 2.592$**
Males	Breeding treatment	1	$F = 55.151$***
	Family	24	$F = 13.444$***
(C) Survival			
	Breeding treatment	1	$\chi^2 = 0.0008$
	Family	25	$\chi^2 = 13.24$
(D) Body size (2001)			
Females	Breeding treatment	1	$F = 4.44$*
	Family	25	$F = 1.6$
	Development time	1	$F = 57.067$***
Males	Breeding treatment	1	$F = 5.9$*
	Family (treatment)	24	$F = 1.948$*
	Developmental time	1	$F = 12.663$**
(E) Body size (2000)			
Females	Breeding treatment	1	$F = 0.119$
	Family	6	$F = 0.649$
Males	Breeding treatment	1	$F = 7.964$**
	Family	5	$F = 1.538$

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

on offspring sex ratio (within patch offspring: 150 females and 118 males; between patch offspring: 136 females and 113 males; $\chi^2 = 0.04$, $P = 0.83$).

Adult body size of F_1 offspring was significantly positively influenced by development time (Table 7). Family origin influenced adult body size as well, whereas no effect of breeding treatment was found on either female size or on the size of males with a short development time (<375 days, Fig. 3C, Table 7). Male offspring with a prolonged development time (>375 days) from between patch matings were larger than those from within patch matings (Fig. 3C).

We found no consistent effect of breeding treatment on the value of fitness traits calculated as inbreeding depression coefficients. Most trait values differed with less than 10% between treatments and there were both positive and negative effects in the different traits.

Relatedness

Twenty-eight TE-AFLP bands were scored for each of 64 spiders used in the patch mating experiments. Five bands were fixed (present in all 64 individuals), the rest were present in at least two individuals and absent in at least two individuals (parsimony informative). No two individuals showed the same phenotype (banding pattern) and genetic distances between pairs of individuals ranged from two to 15.

The presence or absence of 31 bands was scored for the 45 spiders comprising groups of siblings. Of these, five bands were fixed, three were present in only a single individual each, and 23 were parsimony informative. Two pairs of siblings had identical banding patterns, whereas all others had unique phenotypes. Genetic distances among the 45 spiders ranged from 0 to 14.

AMOVA analysis of 60 spiders from 20 patches revealed significant genetic differentiation among patches ($\Phi_{ST} = 0.311$, $P < 0.0005$); patches with only one fingerprinted spider were omitted. In comparison, the AMOVA for 16 groups of full siblings showed that roughly half of the observed genetic variation is explained by differentiation among sibships ($\Phi_{ST} = 0.536$, $P < 0.0005$).

One-way analysis of variance showed significant differences among sibling pairs, patch pairs, and nonpatch pairs ($P = 0$, $F = 16.15$, df = 2.45). Mean genetic distance between sibling pairs (mean genetic distance = 2.95 ± 1.7, $n = 21$ pairs of full sibs, each individual used in only one pairwise combination) was significantly smaller than mean genetic distance between mated pairs from the same patch (genetic distance = 5.3 ± 2.4, $n = 16$ mated pairs, $q = 4.95$, Tukey test critical value = 4.2, $P < 0.01$) or mated pairs from different patches (7.1 ± 2.0, $n = 11$ mated pairs, $q = 7.68$, Tukey test critical value = 4.2, $P < 0.01$). The difference between same-patch and different-patch pairs was not significant (q = 3.16, Tukey test critical value = 3.4 for $P < 0.05$; although the difference was judged significant under the less conservative Student-Newman-Keuls test, critical value = 2.8, $P < 0.05$).

DISCUSSION

Dispersal

The mating dispersal of *S. lineatus* shows two characteristics. First, a high proportion of males made short moves. This dispersal tactic increases the frequency of mating with relatives, because natal philopatry and founder events may result in the formation of kin groups (Lubin et al. 1998; Johannesen and Lubin 1999, 2001). Philopatric dispersal and local mating by males suggest relaxed selection against mating with close kin. Secondly, evidence for outbreeding opportunities includes the long-distance dispersal by a substantial number of immigrant males and later long-distance moves of local males. With short moves, males may secure reproductive success with a low cost of dispersal, whereas movement to more distant females would reduce the likelihood and potential costs of mating with close relatives. Males that mated multiply often used both tactics. In the Karpathos populations, some males mated with the nearest female, whereas others moved very long distances. In the Negev populations, males appeared to first travel to nearby females although their later moves took them further away, which could take them to more distant patches likely to

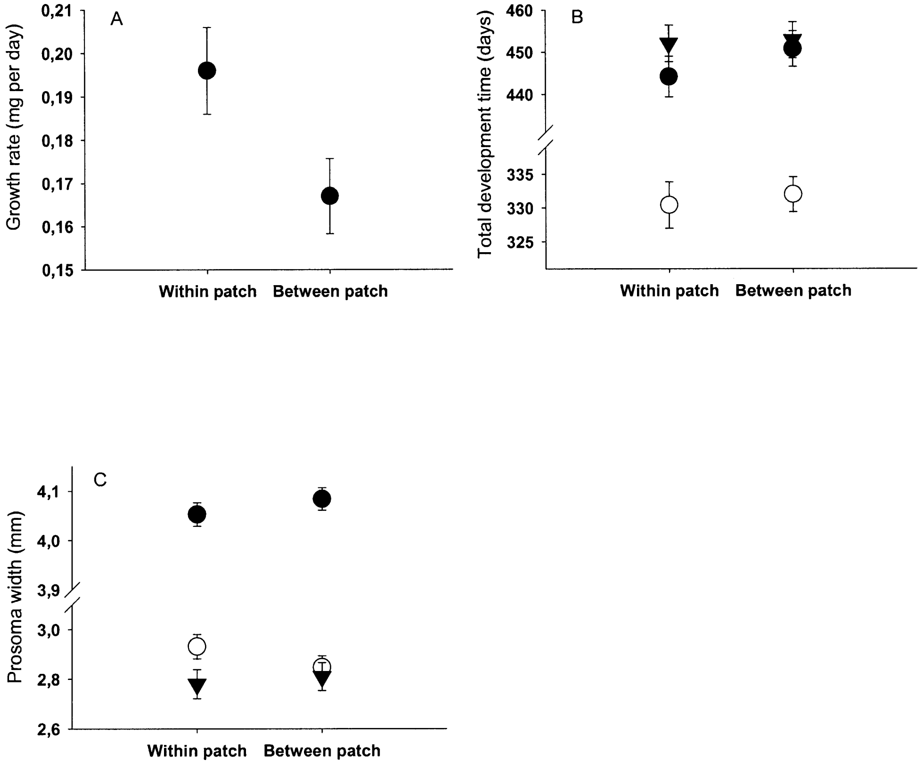

FIG. 3. Offspring life-history traits when *Stegodyphus lineatus* spiders were mated either with mates from their own isolated patch in the field (natural level of inbreeding) or with mates from distant patches (>100 m). (A) Offspring growth rate (mg per day) measured over a two-month period, 235 within patch offspring and 212 between patch offspring; (B) total development time (days) from hatching to adult for 124 within patch and 117 between patch female offspring (●, developmental time < 550 days), 61 within patch and 67 between patch male offspring (○, <375 days), and 55 within patch and 44 between patch male offspring (▼, 375–550 days); (C) adult body size (prosoma width in mm) for 124 within patch and 117 between patch female offspring (●); and 61 within patch and 67 between patch male offspring (○, <375 days), and 55 within patch and 44 between patch male offspring (▼, 375–550 days).

contain unrelated spiders (Johannesen and Lubin 1999, 2001).

Sexual dimorphism with respect to dispersal behavior is expected if the cost of inbreeding is high (Motro 1991) and male-biased dispersal should compensate for female philopatry to reduce the likelihood of inbreeding (Ishibashi et al. 1998; Perrin and Mazalov 2000). Our data only partially corroborated the expected breeding dispersal pattern; we found a relatively high proportion of immigrant males but also a high proportion of philopatric matings. Thus, our data support the suggestion by Johannesen and Lubin (1999; 2001) that restricted male dispersal enhances substructuring within populations of *S. lineatus*.

Inbreeding Experiments

The mating experiments established that *S. lineatus* lacks obvious behavioral mechanisms to prevent mating with close relatives, a result that was consistent for three independent datasets. Thus, we found no effect of relatedness on male courtship behavior, female receptiveness, or mating success, whether mates were full siblings or nonsiblings, patch mates or nonpatch mates. Inbreeding avoidance mechanisms are predicted to evolve when the likelihood of mating with kin is high; however, empirical data lend only partial support for the existence of such a relationship (Charlesworth and Charlesworth 1987; Pusey and Wolf 1996). Furthermore, it is

TABLE 5. The effect of within and between patch matings on mating behavior in 2001. None of the variables differ significantly. Data were log(x + 1) transformed before used in ANOVA. For continuous data, median and range are given in parentheses.

	Within patch mate	*n*	Between patch mate	*n*	Test statistic
Mating pairs	33	41	27	31	*Log-l_3 = 28.159
Aggressive females	12	41	5	31	*Log-l_3 = 36.153
Vibrating males	29	33	25	28	*Log-l_3 = 19.277
Vibration number	5 (0–33)	25	5 (0–19)	32	U = 456.5
Time to enter nest (s)	84.5 (10–3804)	26	148.5 (7–7440)	31	$F_{3,54}$ = 0.864
Time to mate (s)	348 (30–4940)	26	347 (48–7620)	30	$F_{3,53}$ = 0.888
1st mating duration	1005.5 (250–4674)	26	933 (294–5513)	26	$F_{3,49}$ = 0.858

* Log-l stands for the log-likelihood ratio derived from a logistic regression with female and male sizes as covariates.

TABLE 6. ANCOVA of the effect of breeding treatment on fitness variables (mean and SE) of offspring resulting from crosses within patches ($n = 30$) or between patches ($n = 28$). Average juvenile mass is measured as total mass of young/number of young. Statistical results are given for the full model and when significant also for the factor and covariates.

Source	Within patch	Between patch	Test statistics
Clutch size	84.3 (5.12)	86.42 (4.86)	$F_{3,53} = 0.114$
Hatching time (days)^3	26.93 (0.25)	26.96 (0.28)	$F_{3,53} = 0.218$
Hatching percent^1	85.89 (0.02)	88.24 (0.04)	$F_{3,52} = 1.225$
Average juvenile mass (mg)^2	5.06 (0.35)	4.61 (0.29)	$F_{3,53} = 12.702$***
Breeding treatment			$F_{3,53} = 0.679$
Female size			$F_{3,53} = 0.334$
No. of young in clutch			$F_{3,53} = 36.132$***
Days to disperse^4	39.3 (0.88)	39.69 (1.14)	$F_{3,52} = 7.991$**
Breeding treatment			$F_{3,52} = 1.277$
Female size			$F_{3,52} = 0.184$
No. of young in clutch			$F_{3,52} = 21.855$***
No. of dispersing young^5	73.22 (4.89)	80.07 (5.03)	$F_{3,52} = 2.18$*
Breeding treatment			$F_{3,52} = 0.481$
Female size			$F_{3,52} = 2.324$
No. of young in clutch			$F_{3,52} = 4.041$*

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

^{1} Arcsine and ^{2} square root and ^{4} log transformed for analysis.

Female size (prosoma width) was taken as covariate for all analyses. Further covariates: ^{2} clutch size, ^{3} date of egg-laying, ^{4} clutch size, ^{5} hatching date.

difficult to establish whether behaviors that reduce the likelihood of mating with kin result from inbreeding costs or from intrasexual competition or resource competition. Reduction or loss of inbreeding avoidance behaviors may arise if the costs of lost mating opportunities to avoid inbreeding exceed the costs of inbreeding (Waser et al. 1986; Keller and Arcese 1998). Inbreeding could be tolerated, for example, when the risk of mortality during dispersal exceeds the fitness costs of philopatry and inbreeding. However, data on mortality during dispersal are lacking for most species. In *S. lineatus*, indiscriminate mate choice with respect to the degree of kinship and short dispersal distances resulting in within-patch matings suggests that costs of inbreeding are relatively low.

We found support for this hypothesis when we analyzed the fitness consequences of inbreeding by comparing inbred and outbred crosses. We investigated the effects of inbreeding on fecundity and offspring fitness both under high level (full-sib matings) and low level of inbreeding (matings between individuals within isolated patches). Inbreeding depression was detected in the high inbreeding treatment, but the negative effects of full sibling matings were relatively small in the majority of life-history traits investigated. Not all traits were affected; mating with full sibs did not reduce fecundity, hatching percentage, or survival of offspring, but it did cause a reduction in juvenile mass, growth rate, and adult offspring body size.

Inbreeding depression, defined as the decline in the value of a fitness trait, can be measured by the coefficient of inbreeding depression. Full-sib matings in animals may result in a reduction in trait value in the range 10–50% (Roff 1997; 2002). The decline in trait values as a result of full–sib matings in *S. lineatus* were generally less than 10 %, with the exception of juvenile body mass (21%) and growth rate (38%). Life-history traits, which are those most directly related to fitness (Mousseau and Roff 1987), are expected to show relatively high levels of inbreeding depression compared with morphological or other traits (Falconer 1989; Crnokrak and Roff 1995; Roff 1998; DeRose and Roff 1999). This prediction was only partly supported in our study, in which life-history traits such as fecundity, hatching success, and survival declined very little following inbreeding. However, growth rate and juvenile body mass which are also directly related to fitness (Mousseau and Roff 1987; DeRose and Roff 1999) were significantly reduced by inbreeding. The low costs of inbreeding for fecundity and survival in *S. lineatus* suggest a relatively high tolerance to inbreeding at the level of full-sib matings. By contrast, comparable studies of other arthropods commonly demonstrate strong inbreeding depression in fecundity or survival (e.g., Ehiobu et al 1989; Pray and Goodnight 1995; Roff 1998; Van Oosterhout et al. 2000; Keller and Waller 2002; Day et al. 2003; Henter 2003; Radwan 2003).

Inbreeding History

Following inbreeding events, increased homozygosity enables natural selection to purge exposed deleterious alleles (Charlesworth and Charlesworth 1987, 1990; Barret and Charlesworth 1991; Latta and Ritland 1994; Crnokrak and Barrett 2002). But, if effective population size is large and inbreeding therefore occurs at a low rate, the risk of fixation of deleterious alleles should be low (Lande and Barrowclough 1987). Populations that regularly inbreed to some degree may thus be less prone to inbreeding depression (Charlesworth and Charlesworth 1987). Empirical support for such adaptations to inbreeding is reported from natural populations of animals (Shields 1993; dos Santos et al. 1995; Lacy and Ballou 1998); and from experimental manipulations of inbreeding rates, in which lower rates of inbreeding increases fitness compared with higher inbreeding rates (Ehiobu et al. 1989; Latter et al. 1995; Day et al. 2003).

Populations with a history of inbreeding should show inbreeding tolerance, and our data support this prediction. We found no cost of natural levels of inbreeding in the patch-mating experiment, although the relatedness analysis showed

TABLE 7. The effect of within and between patch matings on offspring fitness variables: (A) offspring growth rate (mg per day, $n = 447$, square root transformed), measured over a 2-month period; (B) total development time from hatching to adult (days), data for males were analysed separately for development time <375 days, $n = 128$, and 375–550 days, $n = 99$), data for females ($n = 241$, development time >550 days excluded) were log transformed; (C) probability of survival from hatching to adult ($n = 576$); (D) offspring adult body size (prosoma width in mm, log transformed, 241 females, 128 males <375 days, 99 males >375 days). Probability of survival was analysed by logistic regression and nested ANCOVA was used to analyze growth rate, development time and adult body size. Development time and adult sizes were analyzed separately for males and females, as these differed significantly for the two sexes. In all analyses, family was nested within breeding treatment.

	Source	df	Test statistic
(A) Growth rate			
	Breeding treatment	1	$F = 4.281$*
	Family	53	$F = 1.573$*
	Sex	1	$F = 3.353$
(B) Development time			
Females	Breeding treatment	1	$F = 0.46$
	Family	54	$F = 1.172$
Males (<375 days)	Breeding treatment	1	$F = 0.143$
	Family	46	$F = 3.002$***
Males (>375 days)	Breeding treatment	1	$F = 0.004$
	Family	39	$F = 1.792$*
(C) Survival			
	Breeding treatment	1	$\chi^2 = 0.016$
	Family	53	$\chi^2 = 94.939$**
(D) Adult body size			
Females	Breeding treatment	1	$F = 0.928$
	Family	54	$F = 1.479$*
	Development time	1	$F = 40.006$***
Males (<375 days)	Breeding treatment	1	$F = 0.422$
	Family (treatment)	46	$F = 2.233$**
	Developmental time	1	$F = 29.927$***
Males (>375 days)	Breeding treatment	1	$F = 7.468$*
	Family	39	$F = 1.614$*
	Developmental time	1	$F = 6.1$*

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

a genetic correlation of 30% among spiders within a patch. Genetic similarity among patch mates in this study was significantly lower than that of full siblings, and the two levels of relatedness among spiders tested for inbreeding depression were clearly distinguishable by the genetic fingerprints. However, even with full-sib matings, where inbreeding effects should be maximized, inbreeding depression was relatively mild and affected mainly correlated developmental and morphological traits. It is therefore likely that philopatry and ecological constraints on outbreeding mechanisms in this system exceed the costs of low levels of inbreeding and that inbreeding tolerance has evolved through a history of inbreeding.

Polyandry, Protandry and Inbreeding Avoidance

Polyandry may reduce the risk of inbreeding in situations where mating with close relatives is likely (''inbreeding avoidance hypothesis,'' Stockley et al. 1993; Newcomer et al. 1999). Although natal philopatry and partially restricted male dispersal increase the likelihood of mating with close relatives in *S. lineatus*, polyandry may counteract effects of inbreeding by reducing the risk for a female of using genetically incompatible sperm or by allowing for postmating sperm choice (Zeh and Zeh 1996; Tregenza and Wedell 2002). In our study, one third of the females mated with more than one male and multiple mating seems to occur regularly in this species (Schneider and Lubin 1996, 1998). Lifetime reproductive success is represented by a single clutch; hence any cost of inbreeding will have a large impact on female fitness. However, females did not show any inbreeding avoidance behavior. This suggests that the costs of inbreeding are lower than the risk of remaining unmated or that females have other means to reduce potential costs of inbreeding. Tregenza and Wedell (2002) found that crickets preferentially used the sperm of nonsiblings. If such a mechanism existed in *S. lineatus*, polyandry would be beneficial as a means to reduce inbreeding costs. Females regularly mate multiple times prior to egg production although there are no obvious benefits (Maklakov and Lubin 2004). Following oviposition, females are aggressive towards males because they pay a high cost of having to replace the eggsac if the male can force his interests on her (Schneider and Lubin 1996, 1997). Nevertheless, females that are overpowered by an infanticidal male will resume defense and mate with the male. Although the costs of multiple mating before oviposition may be offset by inbreeding avoidance, additional matings after oviposition are very costly. However, if late arriving males are likely to come from distant patches and therefore are unrelated, mating with these males increases the genetic variation of the offspring and could balance the cost associated with polyandry.

Protandry is also suggested as a mechanism to prevent inbreeding (Morbey and Ydenberg 2001), and may counteract inbreeding due to population subdivision. *Stegodyphus lineatus* males mature on average 16 days earlier than females (Schneider 1997). A lack of mature females in their immediate surroundings could lead males to attempt to find females in neighboring or more distant patches. Because patch mates may consist of siblings (Lubin et al. 1998, Johannesen and Lubin 1999, 2001), emigration would reduce the likelihood of mating with kin. Bukowski and Avilés (2002) suggested that asynchrony in maturation between sexes may limit inbreeding and also explain an apparent lack of discrimination of kin as mates in another subsocial spider, *Anelosimus jucundus* (Theridiidae). Dispersal patterns of *A. jucundus* were similar to *S. lineatus*, because a fraction of males were philopatric and mated locally whereas other males emigrated beyond the local area (Avilés and Gelsey 1998). Thus, an apparent lack of inbreeding avoidance behavior is common for subsocial species of two phylogenetically distant families, each of which also contains regularly inbreeding social species (Avilés 1997).

Evolution of Sociality

The transition to social inbred mating systems is theoretically assumed to be constrained by high costs of inbreeding, which would be reflected in inbreeding avoidance in the subsocial and presumably outbred ancestors. Instead, we show that philopatry, lack of kin discrimination, and restricted male dispersal all contribute to population structuring. The life history and population structure make low-level inbreeding a natural occurrence and the populations are adapted to this level of inbreeding. Consequently, the transition to completely inbred social systems is less constrained by costs of inbreeding depression than one might expect.

The transition to inbred social systems is nevertheless expected to be associated with an initial cost of regular and high levels of inbreeding, which are balanced by benefits of group living. The key to understanding this transition lies in discovering the major causes of selection in favor of group living which includes cooperation in prey capture, brood rearing, and the elimination of a costly dispersal phase. The latter may play a particularly central role in the transition to regularly inbreeding systems (Waser et al. 1986; Perrin and Goudet 2001; Perrin and Lehmann 2001; Burland et al. 2002; Yoder et al. 2004). Regular inbreeding could evolve under conditions where dispersal is ecologically constrained and resource availability or reduced risk of mortality magnifies the benefits of sociality (Waser et al. 1986; Avilés 1997; Perrin and Lehmann 2001).

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