

FITNESS CONSEQUENCES OF OUTCROSSING IN A SOCIAL SPIDER WITH AN INBREEDING MATING SYSTEM

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Inbreeding mating systems are uncommon because of inbreeding depression. Mating among close relatives can evolve, however, when outcrossing is constrained. Social spiders show obligatory mating among siblings. In combination with a female-biased sex ratio, sib-mating results in small effective populations. In such a system, high genetic homozygosity is expected, and drift may cause population divergence. We tested the effect of outcrossing in the social spider *Stegodyphus dumicola*. Females were mated to sib-males, to a non-nestmate within the population, or to a male from a distant population, and fitness traits of F1s were compared. We found reduced hatching success of broods from between-population crosses, suggesting the presence of population divergence at a large geographical scale that may result in population incompatibility. However, a lack of a difference in offspring performance between inbred and outbred crosses indicates little genetic variation between populations, and could suggest recent colonization by a common ancestor. This is consistent with population dynamics of frequent colonizations by single sib-mated females of common origin, and extinctions of populations after few generations. Although drift or single mutations can lead to population divergence at a relatively short time scale, it is possible that dynamic population processes homogenize these effects at longer time scales.

KEY WORDS: Inbreeding depression, inbred sociality, local adaptation, population incompatibility, purging, social spiders.

Most animals appear to have an outcrossing mating system and reduce the likelihood of mating with close relatives through discrimination against kin as mates (Ciszek 2000; Mateo 2003) or sex-biased mating dispersal (Pusey and Wolf 1996; Braude 2000; Perrin and Mazalov 2000; Hazlitt et al. 2006). Consequently, animal mating systems where inbreeding is the rule rather than the exception are rare in nature (but see Waser 1993; Chapman et al. 2000; Peer and Taborsky 2005; Dolgin et al. 2007; Lubin

and Bilde 2007; Thurin and Aron 2009). However, if the costs of avoiding inbreeding are higher than the costs of engaging in inbreeding, mating among close relatives can evolve (Waser et al. 1986; Bilde et al. 2005; Kokko and Ots 2006; Szulkin et al. 2013). These costs include high mortality risks of dispersal, high energetic expenditure associated with the movement itself, increased risk of predation, straying into an unsuitable environment, and difficulties in finding mates (Gwynne 1987; Kokko and Ots 2006;



Lane and Shine 2011). Group living animals in particular may face an elevated challenge of maintaining an outcrossing mating system. Group living and cooperation often evolve by philopatry in response to ecological constraints, for example, lack of vacant breeding territory or high mortality during natal dispersal (Davies et al. 2012). Philopatry and elimination of natal dispersal result in viscous or structured populations composed of family groups (Lion and van Baalen 2008), where group members experience a higher risk of mating with close relatives. Most cooperatively breeding mammals and birds, and the majority of social insects live in family groups, and maintain an outcrossing mating system through sex-biased dispersal. However, a few social animals such as spiders and bark beetles have evolved regular intragroup mating and reproduce with close relatives within their family groups (Bilde et al. 2005; Peer and Taborsky 2005; Kokko and Ots 2006; Bilde and Lubin 2007).

Permanently social spiders are considered to have evolved from solitary ancestors through the elimination of premating dispersal and philopatry (Lubin and Bilde 2007). Social spiders are cooperative breeders with allomaternal care, and they undergo the complete life cycle within a communal nest (Aviles 1997; Lubin and Bilde 2007). New nests are established by individual mated females, broods show highly female-biased primary sex ratio, and offspring mature and reproduce with siblings within the nest. Natal dispersal is completely eliminated, and males rarely leave their nest to seek other mates (Lubin et al. 2009). Nests are usually established on shrubs or trees and consist of a central communal retreat and extended capture webs for the interception of flying insects. Once nests are established, they can give rise to satellite nests through fission, where nests are split into two or more units on the same host plant (Aviles 1997; Bilde et al. 2007; Lubin and Bilde 2007). This form of nest proliferation results in an aggregated distribution of social spider nests within populations. The suite of traits characterizing permanently social spiders have evolved independently multiple times across the spider phylogeny, suggesting that common genetic and ecological factors result in convergent evolution of traits in the social species (Lubin and Bilde 2007; Aviles and Purcell 2012; Majer et al. 2013).

Social spiders go through several generations of repeated mating among siblings within the nest, and group size expands rapidly owing to the female-biased sex ratio. Typically, individual nests as well as entire clusters and populations go extinct after relatively few generations (Aviles 1997; Lubin and Bilde 2007), that is, five to six generations in the annual *Stegodyphus mimosarum* (Crouch and Lubin 2001; Lubin and Crouch 2003). At this stage, mass propagule dispersal of individual mated females is induced, and new nests are founded by their offspring (Seibt and Wickler 1987; Schneider et al. 2001). Successful founders thus establish new populations. Aggregations of nests may go extinct simultaneously, resulting in dramatic boom-and-bust colony dynamics

with frequent extinctions and new colonizations of entire populations (Crouch and Lubin 2001; Bilde et al. 2007; Lubin and Bilde 2007). Repeated intra-nest mating among siblings is expected to result in very high or complete homozygosity (Charlesworth and Charlesworth 1987; Charlesworth and Willis 2009). This is supported by population genetic studies (mainly allozyme or mitochondrial) based on relatively few loci (reviewed in Lubin and Bilde 2007). Most nests have only a single mtDNA haplotype, supporting nest establishment by a single matriline (Johannesen et al. 2002), although propagule dispersal could occasionally facilitate gene flow between populations by long-distance dispersal and the joining of existing nests (Lubin and Crozier 1985; Smith et al. 2009; Johannesen et al. 2009). However, at the population level, genetic homozygosity is expected, as effective population sizes in a system like the social spiders should be very small (Charlesworth 2003). Lethal recessive mutations are quickly purged from such populations, as they are unmasked in the homozygote state (Wang et al. 1999), whereas weakly deleterious recessive mutations may become fixed due to drift and thus accumulate (Charlesworth 1990, 2003; Mattila et al. 2012).

Population studies in selfing plants suggest that small population sizes and limited dispersal result in population divergence and genetic structure, revealing isolation by distance even at very small scale of a few meters (Waser et al. 2000; Crnokrak and Barret 2002). This is manifested in outbreeding depression in offspring of hybrid crosses, suggesting population divergence through drift, or local adaptations leading to population incompatibilities (Waller 1993; Whitlock 2002; Glémin et al. 2006; Dolgin et al. 2007). We aimed to test whether similar processes apply in social spiders, by examining effects of outcrossing in a social spider. We performed controlled mating experiments in the regularly inbreeding social spider *Stegodyphus dumicola* (Eresidae) by crossing females with males from (1) the same nest (sib-mating), (2) a nest within the same population at a distance of at least 50 m away (within-population outcrossing), (3) a different population, 400 km away (between-population outcrossing), assuming increasing population divergence with geographic distance (Smith et al. 2009). If outcrossed offspring show evidence for outbreeding depression, it would suggest that populations are structured and genetically isolated. Meta-population dynamics may however homogenize populations genetically (Pannell and Charlesworth 1999), in which case no difference between inbred and outcrossed offspring is expected. Finally, if nests are less genetically homogeneous than anticipated and there is genetic variation for fitness, inbreeding depression would be revealed in crosses between siblings (Charlesworth and Willis 2009).

We compared fitness correlates of females and F1 offspring among treatments, recording fecundity, hatching success of egg sacs (brood), ratio of hatched to unhatched eggs within a brood, and body mass of the recently hatched young. To examine if

environmental conditions affect the expression of inbreeding or outbreeding depression (review in Kristensen et al. 2003), we divided F1s into two feeding regimes of reduced and normal food diets once the young could catch food on their own. We recorded F1 offspring growth rate and survival to adulthood in the two treatment groups, and contrasted the effect of feeding regime on performance among the mating treatments.

Methods

LIFE HISTORY

Stegodyphus dumicola (Eresidae) is a social spider that is found in southern Africa (Kraus and Kraus 1988). Spiders live in nests containing a few to hundreds of individuals. Nests persist for a few generations, and generations are annual and nonoverlapping. These spiders breed cooperatively and only a portion of females reproduce, but both mothers and helping females feed the young by regurgitation and all females are eventually killed and consumed by the young (Salomon and Lubin 2007). Offspring stay within their natal nest and cooperate within their family group. Primary (Aviles et al. 1999) and secondary sex ratios are female-biased (Lubin and Bilde 2007).

NEST COLLECTION AND ESTABLISHMENT OF EXPERIMENTAL GROUPS

We collected 50 spider nests from two natural populations in Namibia; collection and export permits were issued from the Ministry of Environment and Tourism of Namibia (permit number: 1401/2009, to Y. Lubin). We collected 25 nests from each of two populations: Seeis (population S) (22°33'S, 17°36'E), near Windhoek, and Huttenhof farm (population H) (19°28'S, 17°11'E), near Otavi, approximately 400-km apart. These two populations were sampled near areas shown in Johannesen et al. (2002) as being monomorphic for different mtDNA haplotype families (families A and B). Both populations occur in the thornbush savanna biome (Barnard 1998) and nests were found on thorny bushes such as *Acacia* spp. and other Mimosoideae up to 1.5 m above ground. All nests were brought to a field laboratory located near the Huttenhof population. Nests were taken apart and spiders were separated by sex, and females were further separated according to their body size (adult females have larger body size than sub-adult females, see: Salomon et al. 2008). From each nest we created six groups of four subadult females each and placed them in separate plastic mesh boxes (height 12.5 cm, length 12.5 cm, depth 10 cm) covered with thin cloth that allowed air flow. We kept the boxes outdoors under ambient temperature and natural photoperiod and protected from direct sun and rain. Once spiders produced capture webs we started feeding them once a week with a mixed diet consisting of wild caught insects (moths, Lepidoptera; grasshoppers and crick-

ets, Orthoptera). Insects were caught either with a sweeping net in the grassland or at lights at night. Food was allocated according to a rotating feeding cycle to ensure that all spiders were fed with the same variety of prey, and we monitored fed groups to ensure that spiders were indeed feeding on the insects.

MATING EXPERIMENTS

To assess fitness consequences of inbred compared with outcrossed matings, we conducted mating treatment between females and males of assumed decreasing genetic relatedness, based on the geographical distance between the nests they originated from. Within treatment each cross was repeated twice for each nest ($n = 50$, originating from two populations). Mating between females and males from the same nest were “inbred crosses” (IN, $n = 104$ experimental matings originated from the two populations). Matings between spiders originating from nests up to 50 m apart were considered as “within-population crosses” (WP, $n = 98$ experimental crosses, from two populations), as spatial autocorrelation analysis showed that genetic similarity between spiders drops off when nests are more than 30 m apart (Smith et al. 2009). Mating between individuals from the two populations 400 km apart, populations H and S, were considered “between-population crosses” (BP, $n = 102$ crosses). When the first female in a group moulted to adulthood, a male was added, depending on the mating treatment. Because of difficulties in distinguishing between adult and subadult females in this species (*S. dumicola* females have concealed genitalia: Kraus and Kraus 1988), there may have been more than one adult female during male cohabitation. After 1 week, males were removed from the box, and females were raised until the first egg-sac hatched. The remaining egg-sacs were then removed to ensure that there would be only a single brood in each replicate.

Four months after the start of the experiment, the spiders were brought to the Sede Boqer Campus of Ben-Gurion University at Midreshet Ben-Gurion, Israel. Import permits were issued in Israel from the Israel Nature and National Parks Authority (Permit number: 2009/10249, to Y. Lubin). In Israel, spiders remained in the same mesh boxes in which they hatched and were kept outside in a net house partially protected from sun and sprayed with water sprinklers twice a day (at 10 a.m. and 12 noon). Spiders were fed with a mixed diet of lab reared insects: crickets, *Acheta domestica*; grasshoppers, *Locusta migratoria*; and flies, *Musca domestica*.

FITNESS TRAITS OF OFFSPRING

We examined the groups on a weekly basis and recorded egg-sac production (produced or not), timing of egg-sac production, number of egg-sacs produced, and their hatching success (egg-sac hatched or not). All treatment groups were monitored for at least 6 months, and if none of the egg-sacs hatched, it was considered a failed brood. Once young killed and ate the first female, or

if 60 days after hatching elapsed without the young consuming a female, we counted and weighed the offspring as a group using an electronic balance (accuracy ± 0.1 mg), and removed the hatched egg-sac for further inspection. Hatched egg-sacs were opened under a dissecting microscope and we recorded the number of undeveloped eggs (within a brood), unhatched embryos, and dead first instar spiderlings (young emerge from the egg-sac in their second instar).

To examine the effect of food level on offspring growth rate and survival among treatment groups, we altered the amount of food a group received, so we could unmask any effects of the rearing conditions and spiders' genetic background (Armbruster and Reed 2005). We divided each brood into two equal-sized groups ($N = 10$) 3 to 5 months after hatching (September 2010). Groups were provided either a control or reduced diet, and were fed once every 10 days. If the original brood had fewer than 20 spiderlings, we created only one treatment group receiving the control diet. The control group received at each feeding approximately 0.8 g of prey (6–8 prey items) and the reduced-diet group received half this amount, but the same variety of prey. We weighed the young as a group every 45 days for 7 months (five measurements). After 7 months, we recorded individual body size (prosoma width), body mass, number of remaining spiders in a group, and number of adult females and males.

STATISTICAL ANALYSES

The data were analyzed by mixed-model analysis of variance (ANOVA) with mating treatment (fixed variable, three levels), female population origin (fixed variable, two levels), maternal nest (matriline; random variable, 50 levels), and interactions between mating treatment and population. The F -statistics for the fixed effects were calculated using restricted residual maximum-likelihood and Satterthwaite's method was used to estimate the degrees of freedom (Zar 1999). Post hoc analyses were performed to evaluate differences between mating treatments. Residuals were checked for normality and homogeneity of variance, and to meet ANOVA assumptions data transformation was performed, if needed. The following variables could not be adequately transformed to meet the criteria for parametric testing: the number of first instar spiders and the surviving number of young. These variables were analyzed using the Wilcoxon chi-square test. The proportions of hatched eggs within an egg-sac, and groups with failed broods were compared using generalized linear models (binomial distribution) with maximum likelihood estimation, with the effect of mating treatment, population of female origin and their interaction. Differences in the number of failed brood within mating treatment between populations were estimated using paired contrasts. Spider growth rate and survival was estimated after spiders were allocated to smaller, diet controlled groups. The time of

measurement was included as a random effect and interaction between mating treatment and time of measurement was calculated to estimate differences between treatments over time. Data are shown as mean \pm standard error unless noted otherwise. Standard errors for proportions were calculated using the normal approximation for binomial distribution. Statistical analyses were done in SAS (SAS Institute Inc., Cary, NC) or JMP 9 (SAS institute Inc., Cary, NC) for nonparametric tests and generalized linear models.

Results

EGG-SAC PRODUCTION AND HATCHING SUCCESS

Females from the H population were larger than females from the S population (average prosoma widths, H: 2.9 mm \pm 0.01, S: 2.8 mm \pm 0.01, population: $F_{1,414} = 23.5$, $P < 0.0001$), but within populations, there was no difference between mating treatments in female body size (population \times treatment: $F_{2,414} = 0.99$, $P = 0.4$). Of the 304 groups established, 93% produced egg-sacs. Each group produced on average 1.84 \pm 0.05 egg-sacs (range 1–4 egg-sacs per group). The number of egg-sacs produced per group was similar in all mating treatments, populations, and matrilines (Tables 1, 2). Latency to egg-sac production (days) was shorter for females from the S population with significant variation among matrilines (Table 2 and Fig. 1). The proportion of groups with hatched egg-sacs, excluding groups that did not produce an egg-sac, was significantly lower in BP-crosses compared to the other mating treatments (Fig. 2). The lowest proportion of groups with hatched egg-sacs was found for females from the S population mated with males from the H population (Table 2 and Fig. 2). However, other variables related to the egg-sac stage were not different in the different mating treatments, populations, and matrilines: the time until egg-sacs hatched, the ratio of hatched to unhatched eggs within a brood, and the number of dead first instar young (range 0–28 per group, Tables 1, 2). The number of hatched spiderlings differed significantly between matrilines (Table 2), with a marginally significant interaction between mating treatment and population (Tables 1, 2). The average individual hatchling mass decreased with the number of hatchlings per group (Spearman P 's correlation: $r = -0.5$, $P = 0.0001$), with a significant interaction between populations and mating treatment (Tables 1, 2).

FITNESS TRAITS OF THE OFFSPRING

After assigning the offspring to diet treatments, there was a highly significant effect of the amount of food given per group on the mass of the young, growth rate, survival, and adult body mass and size. Offspring of females from the H population had a higher growth rate than offspring of females originating from the S population, regardless of the mating treatment (population:

Table 1. Measured traits values of fitness variables of three mating treatments: inbreeding, outcrossing within-population, and outcrossing between-populations.

Trait (units)	Maternal population	Number of matriline families (overall <i>N</i>)	Trait values (mean ± SE)		
			Inbreeding	Outbreeding within population	Outbreeding between populations
Number of egg-sacs per group	H	25 (155)	1.9 ± 0.1	1.6 ± 0.1	1.8 ± 0.1
	S	25 (149)	1.8 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
Time to egg-sac hatching (days)	H	25 (97)	49.9 ± 3.6	46.3 ± 2.5	47.8 ± 3.9
	S	25 (82)	53.1 ± 4.7	47.87 ± 4	43.2 ± 5.3
Proportion of hatched eggs (hatched/total eggs)	H	25 (67)	0.8 ± 0.03	0.8 ± 0.04	0.8 ± 0.03
	S	25 (42)	0.8 ± 0.03	0.8 ± 0.05	0.9 ± 0.02
Number of first instar spiders	H	23 (69)	35.5 ± 3.6	30.6 ± 3.2	39.8 ± 4.5
	S	22 (50)	26.5 ± 2.9	34.9 ± 3.4	30.7 ± 5.8
Average hatchling mass (mg)	H	25 (91)	7.9 ± 0.9	7.3 ± 0.71	7.9 ± 0.9
	S	23 (76)	7.4 ± 0.7	8.7 ± 0.82	8.1 ± 0.9

$F_{1,42.8} = 10.38$, $P = 0.0025$; diet treatment: $F_{1,1221} = 282.5$, $P < 0.0001$; matriline: $Z = 4.12$, $P < 0.0001$; time of measurement: $Z = 1.41$, $P = 0.08$). Survival of individuals did not differ between populations, mating treatments, or interaction between time and treatment. However, individuals with the reduced diet had reduced survival (percent survived, reduced diet: $93.1 \pm 0.9\%$; control diet: $95.2\% \pm 0.8$; feeding treatment: $F_{2,1196} = 11.46$, $P = 0.0007$; time of measurement: $Z = 2.25$, $P = 0.025$; and matriline: $Z = 2.14$, $P = 0.01$). There were fewer individuals within a group at the final measurement in the reduced diet ($n = 212$ groups; median, reduced diet: four spiders; control diet: six spiders; Wilcoxon test: $\chi^2 = 14.9$, $P < 0.0001$). A higher growth rate of young from the H population did not translate into a higher average final body mass (population: $F_{1,34.2} = 0.007$, $P = 0.9$) or body size (population: $F_{1,35.3} = 1.5$, $P = 0.2$). However, adult male offspring from the H population were larger than male offspring from the S population (H population: $N = 28$, $1.9 \text{ mm} \pm 0.05$; S population: $N = 27$, $1.6 \text{ mm} \pm 0.05$; population: $F_{1,20.9} = 8.1$, $P = 0.009$). Diet had a significant effect on body mass and size, and young reared on the control diet were heavier and larger than young from the reduced diet (mass: control diet: $71.6 \text{ mg} \pm 0.8$; reduced diet: $51.7 \text{ mg} \pm 0.8$; $F_{1,1071} = 390.9$, $P < 0.0001$; body size: control diet: $2.9 \text{ mm} \pm 0.02$, reduced diet: $2.7 \text{ mm} \pm 0.01$; $F_{2,972.6} = 0.6$, $P = 0.5$). Individuals in larger group had significantly smaller body mass ($F_{1,908.9} = 86.6$, $P < 0.0001$), and body size ($F_{1,921.3} = 13.6$, $P = 0.0002$).

Discussion

We investigated fitness consequences of outcrossing in a social spider, *Stegodyphus dumicola*, where reproduction occurs within the group among highly related individuals. In sessile and highly

inbred organisms, in particular plants, reduced gene flow and genetic structure can apply even on a small scale of only a few meters and may result in population incompatibility or outbreeding depression (e.g., Waser and Price 1994; Waser et al. 2000; Crnokrak and Barrett 2002). We found evidence for population incompatibility in a single trait, when significantly fewer successful egg-sacs were produced in population crosses between the two geographically separated S and H populations compared to within-population crosses. This effect was stronger in crosses where females originated from the S population. Further evidence for population divergence came from the observed differences between females from the two populations in the time lag to egg-sac production. These results suggest the presence of population divergence at large geographical scale (here 400 km) that may result in population incompatibility.

In contrast, we found no effect of outcrossing on fecundity, and no difference in performance in terms of growth rate and survival to adulthood between inbred and outcrossed F1 offspring. These results are expected when populations are genetically homogeneous, which is a likely scenario in populations of social spiders (Lubin and Bilde 2007). Because inbreeding depression is defined relative to the outcrossed trait value, there must be genetic variability for it to manifest (Charlesworth and Charlesworth 1987). Mating among nestmates (siblings) was not associated with detrimental effects compared with outcrossed matings, suggesting very low genetic variation both within and among nests (Aviles 1997; Lubin and Bilde 2007). Similarly, neither of the two outcrossing mating treatments, within-population and between-population, revealed evidence for outbreeding depression in offspring performance. This indicates limited genetic differentiation between spider nests within populations, and also between geographically separated populations. Absence of

Table 2. Test statistics of analyses of fitness variables from within- and between-population crosses with matriline as random effect. Statistically significant effects are shown in bold.

Trait (unit)	Effect	Test statistics	<i>P</i> value
Number of egg-sacs per group	Mating treatment	$F_{2,229} = 0.11$	0.890
	Population	$F_{1,47.4} = 1.91$	0.170
	Mating treatment \times population	$F_{2,229} = 2.75$	0.066
	Matrilines	$Z = 1.45$	0.073
Latency to egg-sac production	Mating treatment	$F_{2,223} = 0.12$	0.120
	Population	$F_{1,47.6} = 7.78$	0.008
	Mating treatment \times population	$F_{2,223} = 0.27$	0.760
	Matrilines	$Z = 3.61$	<0.001
Proportion of hatched egg-sacs (brood)	Mating treatment	$\chi^2_{2,276} = 14.43$	<0.001
	Population	$\chi^2_{1,276} = 4.44$	0.038
	Mating treatment \times population	$\chi^2_{2,276} = 1.58$	0.450
	Mating treatment	$F_{2,223} = 1.27$	0.280
Time to hatched egg-sacs (days)	Population	$F_{1,50.4} < 0.001$	0.970
	Mating treatment \times population	$F_{2,153} = 0.45$	0.640
	Matrilines	$Z = 0.83$	0.200
	Mating treatment	$\chi^2_{2,110} = 0.41$	0.800
Proportion of hatched eggs (embryo + first spiders)	Population	$\chi^2_{1,110} < 0.001$	0.960
	Mating treatment \times population	$\chi^2_{2,110} = 0.62$	0.700
	Mating treatment	$\chi^2_{2,276} = 3.62$	0.160
	Population	$\chi^2_{2,276} = 0.07$	0.790
Number of first instar spiders	Mating treatment	$F_{2,141} = 1.05$	0.350
	Population	$F_{1,53.6} = 3.64$	0.062
	Mating treatment \times population	$F_{2,141} = 2.98$	0.054
	Matrilines	$Z = 2.27$	0.016
Average hatchling mass	Mating treatment	$F_{2,134} = 1.36$	0.200
	Population	$F_{1,49.3} < 0.01$	0.900
	Mating treatment \times population	$F_{2,134} = 4.58$	0.012
	Number of hatchling per group	$F_{1,158} = 48.51$	<<0.001
	Matrilines	$Z = 2.45$	<0.001

genetic variation in these social spider populations conforms to recent colonizations by a common ancestor (Hasting and Harrison 1994; Pannell and Charlesworth 1999). This scenario is consistent with population dynamics with frequent colonizations by single mated females of common origin, and extinctions of populations after few generations (Crouch and Lubin 2001; Bilde et al. 2007).

Populations of small effective size are subject to genetic drift that can lead to population divergence (Charlesworth 2003). This effect may be reinforced in social spiders, because female-biased sex ratio further reduces effective population size and increases effects of drift. This was partly supported by data suggesting that distant populations are genetically differentiated at least for mitochondrial loci (Johannesen et al. 2002). Population incompatibilities could also arise from single mutations favored by differences in the environment (Waser 1993; Hendry et al. 2001), where single genes confer higher fitness in a certain environment (Glémin et al. 2006). There is evidence for karyotype differences among populations in species of the genus *Stegodyphus* (Forman et al.

2010) that might have similar effects (White 1973). Importantly however, these types of effects are expected to be transient, if population dynamic processes with frequent extinctions and colonizations, as discussed earlier, result in genetic homogenization across populations. Thus, although drift or single mutations can lead to population divergence on a relatively short time scale, dynamic population processes are expected to homogenize these effects at a longer time scale. Consistent with this scenario, a continental scale population genetic study of a social and inbreeding *Stegodyphus* species showed high genetic homogeneity and only small-scale isolation by distance (V. Settepani, J. Bechsgaard, and T. Bilde, pers. comm.). We note that our data revealed substantial colony (matriline) effects on F1 offspring growth traits, these may reflect colony age- or size-dependent effects, but genetic effects cannot be excluded.

We found that reduced diet caused lowered offspring growth rate, adult size, and survival, however we found no interaction between diet and crossing treatments on F1 performance. Inbreeding (or outbreeding) depression can be masked under benign

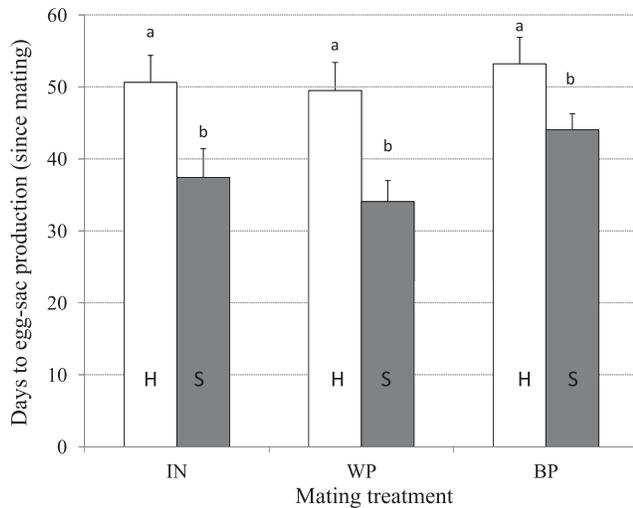


Figure 1. Average (\pm SE) number of days from mating until egg-sac production in three breeding treatments: IN, inbreeding; WP, within-population; and BP, between-population, and two geographical locations: in *white* mothers from H population (letter inside the bar), in *gray* mothers from S population (letter inside the bar). Letters above bars indicate significant differences between populations and crossing treatments (Tukey post hoc tests).

environmental conditions, and reinforced under adverse conditions (Keller and Waller 2002; Kristensen and Sorensen 2005). We tested whether mating treatments might exhibit subtle effects that were only detectable under stressful conditions, but found no evidence for detrimental gene \times environment interactions. However, we found population differences in the response to crossing treatments, in particular, fewer viable egg sacs were produced in between-population crosses, suggesting the existence of subtle population incompatibilities (Peer and Taborsky 2005). Notably, no crossing effects were detected in female fecundity, and once eggs hatched, F1 offspring performed equally well in growth and survival across in inbred and outcrossed treatments, suggesting that depressed egg-hatching success could be a single-gene effect (Glémin et al. 2006).

THE TRANSITION TO INBREEDING

Phylogenetic analyses support the evolution of permanent sociality in spiders by the subsocial route, where groups form by elimination of dispersal which leads to mating among nestmates (Agnarsson et al. 2006; Lubin and Bilde 2007). Permanently social species have evolved from solitary species that often show extended maternal care (subsocal behavior) and prolonged association of offspring in the natal nest. A study of the solitary and subsocial spider *Stegodyphus lineatus* showed limited dispersal and philopatry increases the likelihood of mating with sibs and half-sibs, and that spiders show no behavioral inbreeding avoidance (Bilde et al. 2005). Experimentally inbred and outbred

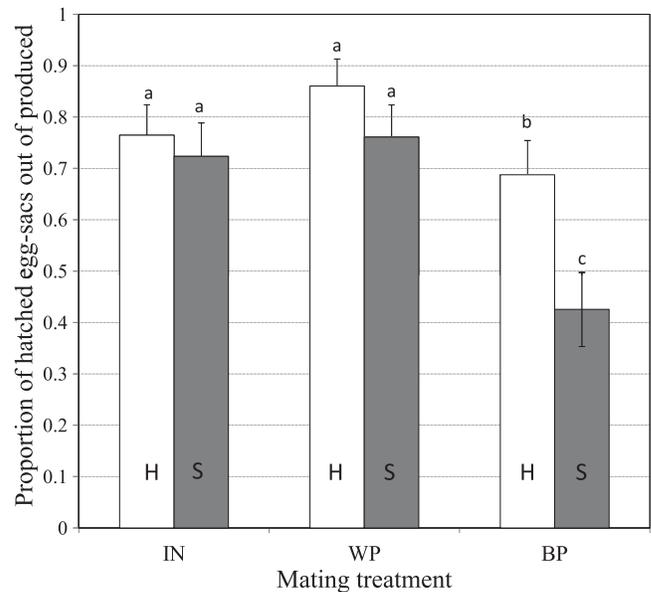


Figure 2. The proportion of groups that produced a hatched egg-sac in three mating treatments: IN, inbreeding; WP, within-population; BP, between-population. Population of origin of the females: in *white*, population H, in *gray*, population S. Letter above bars denote significant differences among levels using contrast tests (see Results).

crosses revealed no or low inbreeding depression in fitness traits measured in the offspring, thus showing high tolerance to inbreeding. In the solitary subsocial spider *Anelosimus jucundus*, fitness costs of experimental inbreeding were detected only in later developmental stages, and are perhaps ameliorated by maternal care and benefits of group living (Aviles and Bukowski 2006). Thus, although both these subsocial species show premating dispersal, and probably outcross frequently, they also exhibit tolerance to inbreeding, suggesting a history of some degree of inbreeding in the population (Charlesworth et al. 1990; Bilde et al. 2005). This likely facilitates the transition to a strict inbreeding mating system in the derived social species, because of the low apparent fitness loss in the transition to inbreeding (Bilde et al. 2005; Aviles and Bukowski 2006; Lubin and Bilde 2007). The absence of short-term costs of inbreeding in permanently social species, as seen in our study, combined with at least occasional mating among relatives and thus low natural levels of mating among relatives in ancestral species (Tuni et al. 2012), conform to the theory that low inbreeding depression is expected in systems with a long history of inbreeding (Husband and Schemske 1996; Escobar et al. 2011). Once costs of inbreeding are overcome, direct and indirect benefits of group living could favor the evolution of permanent sociality and cooperation in groups composed of highly related individuals (Boomsma 2007; Bilde et al. 2007).

Obligatory inbreeding appears to be a relatively rare phenomenon (Wright et al. 2013). In addition, obligatory inbreeding

has been proposed to be an “evolutionary dead-end” because inbreeding lineages suffer from reduced ability to respond to natural selection, increasing the risk of extinction (Stebbins 1974; Wright et al. 2013). There is some support for this scenario in studies of plant genera with mixed mating systems, where selfing lineages are subject to higher rate of extinction (Charlesworth 2003; Wright et al. 2013). Agnarsson et al. (2006) proposed that social inbreeding spiders of the Theridiidae family form phylogenetically isolated clades with high extinction rates, and in the genus *Stegodyphus* Johannesen et al. (2007) suggested that social species have a reduced tendency to diversify. A preliminary genome-wide analysis in *Stegodyphus* indicated elevated nonsynonymous relative to synonymous diversity in a social lineage. This suggests that elimination of deleterious mutations is less effective in social species relative to the solitary subsocial species (Mattila et al. 2012). These results suggest that random genetic drift resulting from reduced effective size and reduced effective recombination rate in small populations might lead to long-term costs through accumulation of weakly deleterious mutants.

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