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No evidence for inbreeding depression and inbreeding avoidance in a haplodiploid mite *Tetranychus ludeni* Zacher

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Mating between relatives (inbreeding) may increase homozygosity of recessive or partially recessive deleterious alleles, resulting in inbreeding depression (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). The cost of inbreeding may drive the evolution of inbreeding avoidance behavior (Pusey & Wolf 1996; Nichols 2017). However, increased homozygosity due to inbreeding could expose recessive deleterious alleles to selection which purges them from the genome (Crnokrak & Barrett 2002; Keller & Waller 2002), resulting in little or no fitness reduction (Nichols 2017). Parents may even gain fitness through inbreeding because mating between relatives helps spread identical beneficial genes by descent (Kokko & Ots 2006; Szulkin *et al.* 2013; Nichols 2017) which will increase fitness (Hamilton 1972; Bai *et al.* 2005). Under these circumstances, animals may not need to avoid inbreeding (Tan *et al.* 2012). Therefore, whether inbreeding avoidance behaviour has evolved in an animal species may depend on the magnitude of inbreeding depression (Lande & Schemske 1985; Szulkin *et al.* 2013; Nichols 2017).

In haplodiploid animals where males are haploid from unfertilised eggs and females are diploid from fertilized eggs, inbreeding depression may be less severe and should only affect female-specific traits such as fecundity and offspring sex allocation (Henter 2003; Mori *et al.* 2005; de la Filia *et al.* 2015; Tien *et al.* 2015) because deleterious alleles are subject to selection in haploid males (Atmar 1991; Antolin 1999; Smith 2000; Henter 2003). However, whether inbreeding avoidance, if any, is sex-specific is unknown. Spider mites are a group of haplodiploid animals where frequent sibling and mother-son mating occurs (Mitchell 1973; Borgia 1980; Avilés & Purcell 2012) because mated mothers often lay haploid and diploid eggs closely together and brothers and sisters develop on the same spot and mate upon emergence (Mitchell 1973). Previous studies show that sibling mating causes no or limited inbreeding depression (Perrot-Minnot *et al.* 2004; Ito *et al.* 2012) whereas mother-son mating leads to substantial depression (Mori *et al.* 2005; Tien *et al.* 2015). However, most studies of effects of inbreeding on reproductive fitness in haplodiploids have only investigated one or a few generations, limiting our understanding of how inbreeding potentially affects long-term fitness.

Here we investigated aspects of inbreeding using the spider mite *Tetranychus ludeni* (Zacher), nothing of which was known prior to this study. We carried out a series of experiments to determine (1) whether and to what extent inbreeding depression occurred in over 11 generations of sibling and mother-son inbreeding and (2) whether the species performed sex-specific inbreeding avoidance.

We established a colony of *T. ludeni* from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand, and reared it on 3- to 5-week-old kidney bean plants (*Phaseolus vulgaris* L.). We then split the colony into two colonies (A and B) and reared them on kidney bean plants in two separate climate rooms for 2.5 months (about 8 generations) before experiments, allowing us to conduct inbreeding and outbreeding treatments (see below). We maintained the colonies and carried out experiments at 25 ± 1°C, 40 ± 10% RH and 14:10 (L:D) photoperiod. We

used the first expanded leaves of 1- to 2-week-old bean plants for all experiments. To prepare mites for inbreeding experiments, we randomly selected 40 male and 40 female deutonymphs from Colony A and maintained them individually until emergence. We allowed newly emerged virgin females to mate with newly emerged virgin males once and then transferred each mated female onto a leaf square (2.0×2.0 cm) placed on wet cotton wool in a Petri dish (9.5 cm diameter \times 1.5 cm height) for oviposition for five days.

To determine whether and how inbreeding affected offspring fitness, we randomly selected three female deutonymphs that developed from the above eggs laid by each female for the following three treatments: (1) MS — mothers mated with their sons for 11 successive generations, (2) BS — brothers mated with their sisters for 11 successive generations, and (3) OB (outbreeding) — females mated with males from Colony B for 11 successive generations. As females in treatment MS were about 10 days old when their sons developed to adults, we used 10-day-old females for all three treatments in each generation to keep female age and oviposition experience consistent. In each generation we individually transferred female deutonymphs prepared as described above onto leaf squares (2.5×2.5 cm) for emergence. We allowed virgin females to reproduce for 10 days and then paired each of them with a newly emerged virgin male according to treatments until death. The leaf squares were replaced once every five days for each replicate. From each mated female, one to three female deutonymphs produced within five days after mother mating was randomly selected to start the next generation. We recorded the lifetime number of eggs laid, offspring survival, offspring sex ratio after mating, and longevity for each pair in the first and 11th generations. We obtained 30, 29 and 31 replicates in the first generation and 28, 27 and 29 replicates in the 11th generation for treatments MS, BS and OB, respectively.

To test inbreeding avoidance behaviour, we used offspring from the 11th generation of the above experiment and carried out two experiments: (1) female mate choice — a female was allowed to choose between a brother and a male from Colony B, and (2) male mate choice — a male was allowed to choose between a sister and a female from Colony B. Female and male mate choice were tested for each of the MS, BS, and OB treatments, resulting in 6 combination choice treatments with 39–56 replicates for each treatment. To start the experiments, we introduced two virgin 1-d-old mates on a leaf square (1×1 cm) and then the test virgin 1-d-old female or virgin 1-d-old male at a point with the same distance from the two mates. We video-recorded each replicate for 15 minutes and recorded premating period, mating success, and mating duration. Mating was scored as successful when the male genital was connected with the tip of the female abdomen for over 30 seconds, during which time insemination occurs (Potter & Wrensch 1978).

The distribution of all data was tested using a Shapiro-Wilk test (UNIVARIATE Procedure) before analysis. Data on the number of eggs and daughters, male and female longevity in the inbreeding experiment, and premating duration in the female mate choice experiment were normally distributed and analysed using an analysis of variance (ANOVA, GLM Procedure) with a Tukey test for multiple comparison. Data on proportion of daughters and offspring survival in the inbreeding experiment, and premating period and mating duration in the male mate choice experiment, and mating duration in the female mate choice were not normally distributed and analysed using non-parametric ANOVA (GLM Procedure). Data on mate choice were analysed with a Chi-square test (FREQ Procedure). We conducted all analyses using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC).

We show that offspring from MS, BS and OB had similar fitness in the first and the 11th generations (Table 1), suggesting that neither mother-son mating nor sibling mating causes inbreeding depression in *T. ludeni* in both short and long term inbreeding. Similarly, Ito *et al.* (2012) report that sibling mating does not trigger inbreeding depression in *T. kanzawai* (Kishida) and Mori *et al.* (2005) reveal that mother-son mating results in no inbreeding depression in most tested

populations of *Stigmaeopsis miscanthi* (Saito). The phenomenon could result from purging of deleterious alleles through haploid males (Atmar 1991; Antolin 1999; Henter 2003; Tien *et al.* 2015) and frequent inbreeding (Mitchell 1973) in haplodiploid mites. However, *T. urticae* (Koch) females suffer substantial inbreeding depression (Tien *et al.* 2015).

TABLE 1. Effects of inbreeding on reproduction and survival of *T. ludeni* in different generations.

Treatment	Number of eggs	Number of daughters	Daughters (%)	Offspring survival (%)	Longevity (days)	
					Female	Male
First generation						
MS	75.73 ± 6.78	36.50 ± 4.15	75.19 ± 2.15	92.29 ± 0.94 ab	22.59 ± 0.86	30.52 ± 1.57
BS	69.76 ± 4.48	35.24 ± 3.18	80.82 ± 1.84	91.57 ± 0.82 ab	22.07 ± 0.93	28.08 ± 1.52
OB	68.13 ± 4.37	34.26 ± 3.66	78.61 ± 1.92	93.83 ± 0.69 a	20.81 ± 0.63	26.96 ± 1.58
Eleventh generation						
MS	83.11 ± 6.98	40.50 ± 3.79	71.60 ± 3.16	87.54 ± 1.44 b	24.63 ± 1.33	24.58 ± 2.49
BS	72.89 ± 5.22	37.89 ± 3.88	73.52 ± 3.28	86.50 ± 3.06 b	22.89 ± 1.33	23.23 ± 2.77
OB	83.38 ± 5.70	47.21 ± 3.65	77.02 ± 1.64	88.25 ± 1.17 b	24.41 ± 1.04	24.41 ± 1.69
F _(df)	1.1 _(5,168)	1.63 _(5,168)	1.68 _(5,168)	5.6 _(5,168)	1.86 _(5,163)	1.93 _(5,154)
P	0.364	0.1536	0.1414	< 0.0001	0.104	0.0924

MS, mother-son mating; BS, brother-sister mating; OB, outbreeding. Means (± SE) of offspring survival (%) with the same letters are not significantly different (non-parametric ANOVA: P > 0.05).

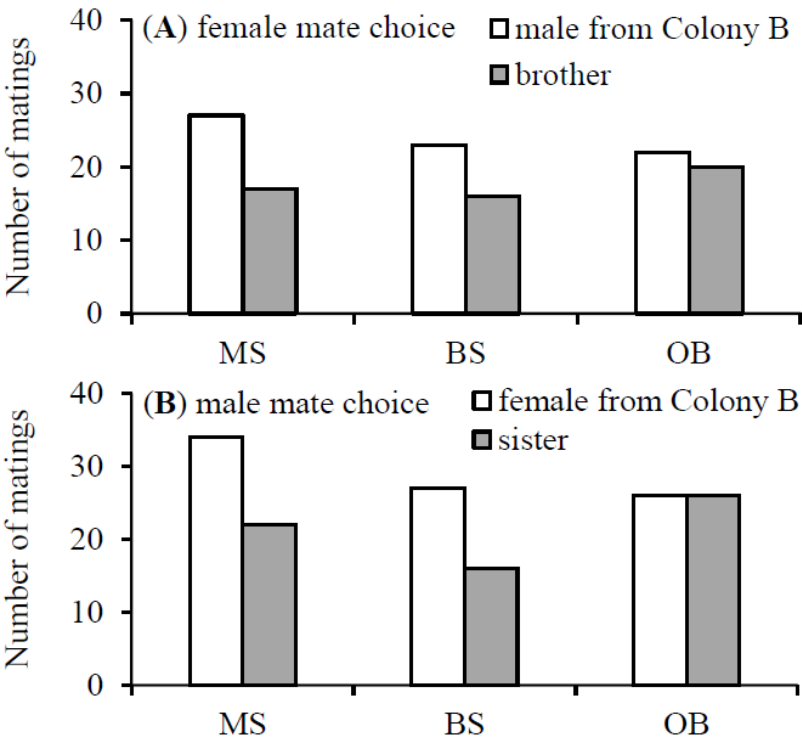


FIGURE 1. Female (A) and male (B) *Tetranychus ludeni* mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating.

Theory predicts that individuals may avoid inbreeding when inbreeding depression is substantial but inbreeding avoidance may not occur when inbreeding depression is low or absent (Lande & Schemske 1985; Szulkin *et al.* 2013; Nichols 2017). Indeed, inbred *T. ludeni* had no significant preference between siblings and unrelated mates in mate choice (For females: MS, $\chi^2 = 2.27$, $P = 0.1317$; BS, $\chi^2 = 1.26$, $P = 0.2623$; OB, $\chi^2 = 0.10$, $P = 0.7576$, Figure 1A; for males: MS, $\chi^2 = 2.57$, $P = 0.1088$; BS, $\chi^2 = 2.81$, $P = 0.0934$; OB, $\chi^2 = 0.00$, $P = 1.0000$, Figure 1B). Furthermore, when mated with siblings or unrelated mates, *T. ludeni* had similar premating period (For females: $F_{5,119} = 1.18$, $P = 0.3229$, Figure 2A; for males, $F_{5,145} = 1.98$, $P = 0.0842$, Figure 2B) and mating duration (For females: $F_{5,119} = 0.24$, $P = 0.9429$, Figure 2C; for males, $F_{5,145} = 0.24$, $P = 0.9444$, Figure 2D). Our findings suggest that both sexes of *T. ludeni* do not avoid mating with kin at all inbreeding levels. Lack of inbreeding avoidance has also been reported in several other haplodiploid species (Bourdais & Hance 2009; de Souza *et al.* 2017; Bogo *et al.* 2018). However, *T. urticae* females prefer to mate with unrelated males (Tien *et al.* 2011) due to substantial inbreeding depression that occurs in this species (Tien *et al.* 2015).

In summary, we have found no evidence for inbreeding depression over eleven generations of sibling or mother-son mating in *T. ludeni*. However, our results do not support the prediction that parents may gain fitness through inbreeding. Due to lack of inbreeding depression neither sex of this species displays inbreeding avoidance behaviour in mate choice.

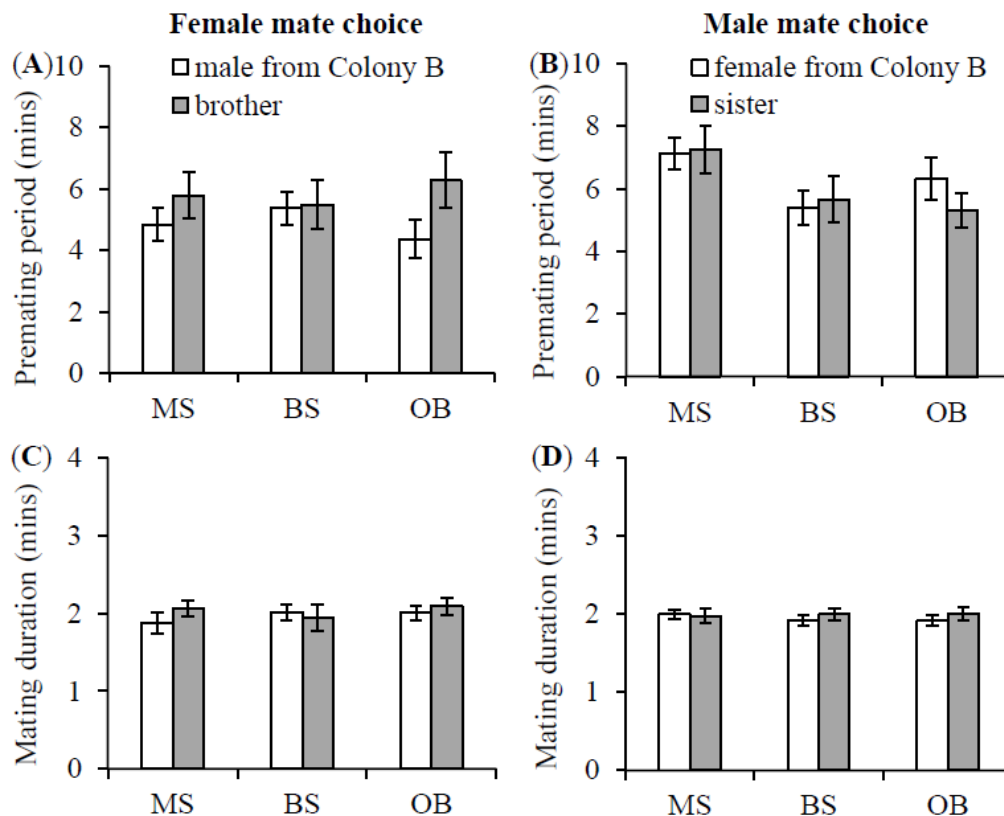


FIGURE 2. Premating period in female (A) and male (B) *Tetranychus ludeni* mate choice, and mating duration in female (C) and male (D) mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating. Error bars are SE.

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