In- and Outbreeding Effects in the Predatory Mite

Phytoseiulus persimilis

Dissertation

Submitted by

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1 General introduction to the thesis

1.1 Rationale

1.1.1 In- and out-breeding

Inbreeding is mating with close relatives (Waser, 1993). Generally, the detrimental effects of inbreeding on reproductive success have been widely documented in numerous vertebrate and invertebrate animals and are almost dogma (Bateson, 1983; for review Charlesworth and Charlesworth, 1987, 1999; Shields, 1993). However, many scientists, especially animal ecologists, argue that inbreeding occurs rarely in nature because individuals commonly avoid mating with their closest relatives (Shields, 1993). To avoid close inbreeding, organisms have evolved various mechanisms such as dispersal from the natal site, extra pair/extra-group copulations, kin recognition, and delayed maturation or reproductive suppression (Pusey and Wolf, 1996). Despite all these mechanisms, inbreeding can occur in small fragmented or isolated populations, which are found in many species (for review Charlesworth and Charlesworth, 1987, 1999) including haplo-diploid plant-inhabiting predatory mites (Acari: Phytoseiidae) (Poe and Enns, 1970; Hoy, 1977). Inbreeding can often lead to detrimental effects in survival, mating, development and reproductive success, intraspecific competitive ability, and/or tolerance of environmental stressors (Crnokrak and Roff, 1999; Hedrick and Kolinowski, 2000; Keller and Waller, 2002; O´Grady, 2006; Charlesworth and Willis, 2009; Fox and Reed, 2011). Organisms can even be exposed to chronic inbreeding in some conditions (Werren, 1993). At the genetic level, inbreeding depression commonly results from increased homozygosity of recessive deleterious alleles (for review Charlesworth and Charlesworth, 1987, 1999; Charlesworth and Willis, 2009). Inbreeding has costs for a variety of reasons such as deleterious alleles can be fully expressed, beneficial interactions (heterozygosity advantage) between different alleles at the same genetic locus can be lost, offspring of inbred parents are more like each other, and reduction or loss of allelic diversity (Bateson, 1983). At the other pole, also mating between genetically distant partners of the same species may be disadvantageous for several reasons such as breaking-up of co-adapted gene complexes by recombination, easier infection by pathogens because of lacking resistance development and the risks and costs associated with travelling between populations (Bateson, 1983; Lynch, 1991). Empirical evidence of outbreeding depression is scarce as compared to inbreeding depression (Edmands, 2007; Frankham et al., 2011).

1.1.2 Study animals

My studies were concerned with in- and out-breeding in the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). These mites are patchily distributed, group-living predators that are specialized on herbivorous spider mite prey such as two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae). *Phytoseiulus persimilis* are haplo-diploid; both females and males arise from fertilized eggs, but males lose the paternal chromosome set during embryogenesis, making them haploid (dubbed pseudo-arrhenotoky; Sabelis and Nagelkerke, 1988 or, more general, paternal genome elimination; de la Filia et al., 2015).

Phytoseiulus persimilis are widely used in biological pest control because of killing and eating unwanted pest mites and their ability to maintain pest populations at non-damaging levels. *Phytoseiulus persimilis* are commercially mass-reared and released in agricultural crops (McMurtry and Croft, 1997; van Driesche and Hoddle, 2009).

Two-spotted spider mites *T. urticae* are herbivorous crop pests in many agricultural settings, present worldwide on a wide variety of plants, including fruits, cotton, vegetables, and ornamentals (Helle and Sabelis, 1985). These herbivorous mites can cause significant yield losses (Van Leeuwen et al., 2007). *Tetranychus urticae* feed by penetrating the plant tissue with their stylets and sucking out cells within the leaf mesophyll. Feeding by spider mites causes graying or yellowing and necrotic spots on leaves; by withdrawing plant nutrients, spider mite feeding slows down plant growth and may lead to defoliation (Xu, 2004).

Figure 1: Adult *Phytoseiulus persimilis* (on the front) feeding on two-spotted spider mite *Tetranychus urticae* (© Peter Schausberger).

Common methods to manage spider mites are still based on the use of synthetic acaricides and insecticides (Van Leeuwen et al., 2010). The repeated and frequent use of insecticides and acaricides can lead to resistance development in *T. urticae* (Yorulmaz and Ay, 2009; Van Leeuwen et al., 2010), since the spider mites have a short life cycle, high reproductive rate, and arrhenotokous reproduction (Yorulmaz and Ay, 2009). Two-spotted spider mites have developed resistance to many insecticides and acaricides and are considered as one of the ´most resistant species` worldwide (Whalon et al., 2008). The use of natural pesticides such as natural enemies has ever increased in the last decades, but its overall proportional share among pest management techniques is still quite low (Katsvanga and Chigwaza, 2004). Synthetic chemical compounds can negatively affect also non-target organisms (also the natural enemies of the pests) and ecosystems (Van Leeuwen et al., 2010). The half-life of some synthetic pesticides in nature can take many years. For instance, parathion, which is one of the most widely used of the organophosphate insecticides and acaricides, has been found to have a half-life of 60 to 80 days on citrus trees (Carson, 1962). Additionally, some pesticides may negatively affect human health. Organophosphorus pesticides (e.g. chlorpyrifos, which is a chemical compound also used against *T. urticae*) commonly accumulate in adipose tissues in liver and kidneys and can consequently lead to serious health problems (Carson, 1962; Şahin et al., 2017). For example, Şahin et al. (2017) examined breast milk samples from mothers living for 5 years in agriculturally used areas and found that organophosphorus pesticides can be detected in breast milk longer than 5 years following pesticide exposure. Changing of host plant nutritional regime is a cultural technique to manage spider mites. Nitrogen fertilization increases the vegetative growth of plants (Spayd et al., 1993). However, deficiency of potassium or large quantities of nitrogen in the host plant tissue can enhance population growth of *T. urticae* (Sabelis, 1986), since nitrogen excess leads to more sensitive leaf tissue. Hence, damage by spider mites and other herbivores is more often seen on plants fertilized by high nitrogen amounts (Altieri and Nicholls, 2003).

1.1.3 Study questions

In contrast to diplodiploid organisms, in- and outbreeding depressions have been rarely studied in haplodiploid organisms. Peer and Taborsky (2005) observed that haplodiploid Ambrosia beetles (*Xylosandrus germanus*) suffered from reduced hatching rates in outbred lines. For the mite family Phytoseiidae, one study was performed by Poe and Enns (1970), who observed high mortality in all life stages, low viability and reduced fecundity in inbred females of laboratory colonies of *Neoseiulus fallacis* and *P. persimilis*. On the other hand, Hoy (1977) reported that nine generations of chronic inbreeding resulted in mild inbreeding depression in the predatory mite *Galendromus occidentalis*. Inbred females of this predatory mite had shorter longevity and lower egg production than outbred females. In my thesis, I addressed the effects of in- and out-breeding in *P. persimilis*, using three levels of genetic relatedness between mates, at both the individual and population level. My thesis aimed at providing a key example of in- and outbreeding in a haplodiploid organism. The results may contribute to optimizing the use of *P. persimilis* in agricultural settings, which represents an ecologically sustainable control method of two-spotted spider mites, and minimizing the risk of environmental problems.

1.2 References

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1.3 The thesis

This thesis consists of three separate but related experimental studies, each represented by a distinct publication. The studies aimed at examining (i) the effects of in- and out-breeding at the individual level, (ii) in- and out-breeding founder effects on growth and dynamics of inbreeding lineages over time, and (iii) female mate choice at different levels of genetic relatedness of their mating partners. All studies were conducted in the laboratory under conditions of 16:8 h L:D photoperiod, 25 ± 1 °C, and 65 ± 5 % RH, using detached bean leaf arenas, acrylic tile arenas and acrylic cages. In all experiments, two-spotted spider mites *T. urticae* were used as prey. *Phytoseiulus persimilis* came from two distinct laboratory-reared populations, called Greece and Sicily. One population was founded by specimens collected in Sicily and the other by specimens collected in Greece. The laboratory populations were each founded by 50 to 100 specimens. The populations were maintained in the laboratory for about 6 to 7 years before use in the experiments.

In the first study, entitled ´´ **Balancing in- and out-breeding by the predatory mite *Phytoseiulus persimilis*``** (Atalay and Schausberger, 2018), I assessed in- and out-breeding depression in predatory mites *P. persimilis* at the individual level using iso-female lines of two populations, from Sicily and Greece. I examined the fecundity of females, and the sex ratio of their offspring, that mated with either a sibling male, a male from the same population or a male from the other population. Additionally, two important components of mating behavior, mating latency and mating duration, were assessed. Firstly, I started the pre-experimental phase by withdrawing gravid females randomly from the rearing units and placing them for oviposition on a fresh detached bean leaf arena with spider mites. Eggs of these females were collected daily and singly transferred onto new arenas and reared there until the deutonymphal stage. Deutonymphal females were collected and isolated in acrylic cages with spider mite prey. After reaching adulthood, females and males were paired to create iso-female lines (dubbed families). Offspring of these pairs were used in experiments.

Figure 2: Fertilized adult female of the predatory mite *Phytoseiulus persimilis* (© www.omafra.gov.on.ca/english/crops/facts/14-013.htm).

To assess the fitness effects of in- and out-breeding, I investigated three levels of genetic relatedness between mates (close - siblings, intermediate – individuals from the same population but not siblings, and distant – individuals from different populations). To control for familiarity effects in sibling matings, offspring from each family grew up separately in two groups; in the experiment, females were then paired with unfamiliar siblings from another group. Virgin females from a given family were paired with either a male from the same family (sibling), or a male from another family from the same population (within population), or a male from a family from the other population (between population) inside acrylic cages, which had been previously loaded with surplus spider mites as prey. Subsequently, the cages were checked every 20 min to record the latency until mating occurred and the mating (copulation) duration. Pairs stayed together in acrylic cages with spider mites until the next day. Next day, I transferred the mated females onto fresh leaf arenas infested by spider mites and recorded daily the number of laid eggs, which were then removed and stored inside closed acrylic cages in the fridge at 8 °C. Eggs laid over five days were taken out from the fridge, placed onto fresh leaf arenas infested by spider mites and allowed to develop to adulthood to determine their sex ratio.

Figure 3: The copulation of adult female (on the left) and male (on the right) of *Phytoseiulus persimilis* (© Michael Seiter).

Females mating with a male from the same population produced more eggs over a longer time than females mating with a sibling or females mating with a male from the other population. Mating duration was disproportionally long in sibling couples. Mating latency was unaffected by mate relatedness. Overall, this study revealed that both close inbreeding and distant outbreeding lead to reduction in fitness traits and suggests that an intermediate level of mate relatedness provides for an optimal balance of genetic relatedness.

In the second study, entitled **”Founder effects on trans-generational dynamics of closed inbreeding lineages of the predatory mite *Phytoseiulus persimilis*”** (Çekin and Schausberger, 2019), I investigated the effects of founder relatedness on growth and development of closed, and thus inbreeding, lineages of the Greece and Sicily populations over four generations. Founder relatedness was established by the same procedure as in the first study, i.e. by pairing a virgin female with either a sibling male, a male from the same population or a male from the other population. The foundresses were transferred onto acrylic arenas with predefined standardized amounts of mixed spider mite stages (brushed from three similarly sized bean leaves in 3 d intervals). Over 40 days, the number of mobile life stages of the predators (juveniles, adult females and adult males) was recorded in 5 d intervals.

For analyses, the results were divided into two periods, the F_1 generation and F_{2-4} generations. In the short-term (one generation), females having intermediate founder mate relatedness (i.e. females mating with a male from the same population), produced more offspring than closely inbred and distantly outbred females, as expected from the first experiment. In the medium- to long-term (F_{2-4}), lineages from both populations, Greece and Sicily, founded by females that had mated with a male from the other population, i.e. distantly outbred females, performed the best, probably because of introducing higher allelic diversity.

This study indicates that the level of founder genetic relatedness plays a decisive role for the dynamics of closed inbreeding populations. Limited prey availability was a stress factor during population development from the F_2 generation onwards. Higher frequencies of deleterious recessive alleles and homozygosity made lineages founded by inbred individuals more sensitive to stressful conditions than lineages founded by outbred individuals.

In the third study, entitled ”**Plastic female choice to optimally balance (k)in- and out-breeding in a predatory mite“** (Schausberger and Çekin, 2019), I examined the mate choice of predatory mite females *P. persimilis* from two different populations, Sicily and Greece. Based on the results of the first study showing that both close inbreeding and distant outbreeding may negatively affect fitness (Atalay and Schausberger, 2018), this study addressed the question whether females can adaptively and flexibly adjust mate choice in dependence of the mate options. As mentioned above, organisms may have evolved several mechanisms to avoid mating with closely related individuals such as kin recognition. Taking that into consideration, I performed three experiments on mate choice, based on the combined predictions of optimal outbreeding and (k)in breeding theories, in predatory mites *Phytoseiulus persimilis*. To assess the mating preferences, females were subjected to binary choice experiments. As in the first two studies, the genetic relatedness of mates was either a sibling, a male from the same population or a male from the other population. Siblings were either familiar or unfamiliar to the female. The experiments were started by giving single females a choice between two males differing in familiarity status or genetic relatedness to the female. To distinguish the two male mate options, their dorsal shields were marked with blue and red water color dots before use in the experiment. In all three experiments and choice scenarios, all females and males were inexperienced regarding the other sex and used only once. In the first experiment, single virgin females were given a choice between a familiar and an unfamiliar brother to assess whether they are able to use direct familiarity (based on prior association) to recognize siblings and avoid mating with them. Females of Greece but not Sicily preferred unfamiliar brothers over familiar brothers. In the second experiment, mate choice options of females were either an unfamiliar or a familiar sibling and a distant kin male (i.e. a male from the same population but not a sibling). Females of both origins, Greece and Sicily, mated preferentially with distant kin than brothers (independent of brothers being familiar or unfamiliar). The third experiment aimed at determining whether *P. persimilis* females are able to discriminate siblings from non-kin males (i.e. males from another population). Siblings were either familiar or unfamiliar to the female. Females from the Greece population preferred to mate with their unfamiliar but not familiar brother, while females of Sicily always preferred brothers (both familiar and unfamiliar) over non-kin males. Overall, this study suggests that females flexibly base their mate preferences on the available mate options and achievable direct and indirect fitness benefits;

they prefer to mate with a male from the same population over brothers but prefer to mate with brothers over non-kin. Regarding the kin recognition mechanisms, Greece females apparently used both direct familiarity (also called recognition based on prior association) (experiment 1) and indirect familiarity (phenotype matching) (experiments 2 and 3), while Sicily females just used indirect familiarity.

1.4 Publications included in thesis

Atalay, D., & Schausberger, P. (2018). Balancing in-and out-breeding by the predatory mite *Phytoseiulus persimilis.* Experimental and Applied Acarology, 74(2), 159-169. doi: 10.1007/s10493-018-0225-3.

Çekin, D., & Schausberger, P. (2019). Founder effects on trans-generational dynamics of closed inbreeding lineages of the predatory mite *Phytoseiulus persimilis*. PloS ONE, 14(4), e0215360. doi:10.1371/journal. pone.0215360

Schausberger, P., & Çekin, D. (2019). Plastic female choice to optimally balance (k)in- and out-breeding in a predatory mite. Behavioural Processes, under review.

2 Publications

Versions presented here represent the versions upon acceptance (publications 2.1, 2.2) and submitted for review (publication 2.3).

2.1

Balancing in- and out-breeding by the predatory mite *Phytoseiulus persimilis*

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Abstract

In- and out-breeding depressions are commonly observed phenomena in sexually reproducing organisms with a patchy distribution pattern, and spatial segmentation and/or isolation of groups. At the genetic level, inbreeding depression is due to increased homozygosity, whereas outbreeding depression is due to inferior genetic compatibility of mates. Optimal outbreeding theory suggests that intermediate levels of mate relatedness should provide for the highest fitness gains. Here, we assessed the fitness consequences of genetic relatedness between mates in plant-inhabiting predatory mites *Phytoseiulus persimilis,* which are obligatory sexually reproducing but haplo-diploid. Both females and males arise from fertilized eggs but males lose the paternal chromosome set during embryogenesis, dubbed pseudo-arrhenotoky. *Phytoseiulus persimilis* are highly efficacious in reducing crop-damaging spider mite populations and widely used in biological control. Using iso-female lines of two populations, from Sicily and Greece, we assessed the fecundity of females, and sex ratio of their offspring, that mated with either a sibling, a male from the same population or a male from the other population. Additionally, we recorded mating latency and duration. Females mating with a male from the same population produced more eggs, with a lower female bias, over a longer time than females mating with a sibling or with a male from the other population. Mating latency was unaffected by mate relatedness; mating duration was disproportionally long in sibling couples, likely indicating female reluctance to mate and sub-optimal spermatophore transfer. Our study provides a rare example of in- and out-breeding depression in a haplo-diploid arthropod, supporting the optimal outbreeding theory.

Key words: *Phytoseiulus persimilis*, predatory mites, inbreeding depression, haplodiploidy, outbreeding depression

Introduction

Inbreeding, the mating or breeding between genetically closely related individuals is common in small, fragmented or isolated populations (for review Charlesworth and Charlesworth 1987, 1999; Frankham et al. 2002; Henter 2003). Inbreeding commonly causes reduction in fitness-related measures such as survival, development, growth rates, fecundity, intraspecific competitive ability, and/or tolerance of environmental stressors (Wright 1977; Henter 2003; Radwan 2003; Fox and Reed 2011). Inbreeding can also lead to sperm deformities, sterility and decreased courtship frequencies (Pusey and Wolf 1996). At the genetic level, inbreeding depression is associated with increased homozygosity. Two major hypotheses of the causal mechanisms of inbreeding depression have been put forward, i.e. the partial dominance hypothesis and the over-dominance hypothesis (Charlesworth and Charlesworth 1999; Wright et al. 2008; Charlesworth and Willis 2009). Both hypotheses assume that increased homozygosity reduces fitness. The partial dominance hypothesis states that fitness reduction arising from inbreeding depends on the genetic load of recessive deleterious alleles. Inbreeding increases the expression of deleterious recessive alleles by increasing the frequency of deleterious homozygous combinations (Waller 1993; Shultz and Willis 1995). The overdominance hypothesis states that heterozygotes generally have a superior fitness than either homozygote (Charlesworth and Willis 2009).

Assuming overdominance as causal mechanism, inbreeding depression should not differ between diplo-diploid and haplo-diploid organisms. Assuming partial dominance, inbreeding depression should primarily occur in diplo-diploids rather than haplo-diploids. In haplo-diploids, in which fertilized eggs commonly become diploid females and unfertilized eggs become haploid males, deleterious recessive alleles should be expressed and purged in haploid males (Lande and Schemske 1985; Charlesworth and Charlesworth 1987). In haplo-diploid species, recessive deleterious alleles are exposed to continuous selection in hemizygous (haploid) males, which are thus expected to carry a lower genetic load (Charlesworth et al. 1990; Wang et al. 1999). Nonetheless, albeit at large and averaged across organisms, inbreeding depression is less severe in haplo-diploids than in diplo-diploids (Antolin 1999; Henter 2003), quite a few studies have shown that excessive inbreeding may also cause significant negative effects in haplo-diploids. For example, in parasitoid wasps inbreeding may cause 38% shortening of longevity and 32% lower offspring production (Henter 2003). Similarly, Whitehorn et al. (2009) observed that inbred haplo-diploid bumblebees suffered from reduced growth rates, shortened longevity and lower offspring production.

As compared to inbreeding depression, also mating between genetically distant individuals may be disadvantageous for a variety of reasons, which is accordingly dubbed outbreeding depression (Bateson 1983; Templeton 1986; Pusey and Wolf 1996). Although outbreeding is commonly related to increased fitness relative to excessive inbreeding, it very much depends on the level of the mates' genetic dissimilarity. Above a critical threshold of genetic dissimilarity of outbreeding mates - in the most extreme case from different species - fitness may be lower than at intermediate levels of genetic dissimilarity (Mitton 1993). Compared to inbreeding depression, experimental documentation of outbreeding depression is scant (e.g. Thornhill 1993; Waser et al. 2000; Peer and Taborsky 2005). The underlying mechanisms of outbreeding depression include underdominance (also called negative overdominance) and deleterious epistatic interactions, resulting in break-down of individual co-adapted gene complexes or, in individuals adapted to local conditions, maladaptation (Charlesworth and Charlesworth 1987; Edmands 2002). Besides genetic costs, outbreeding animals may incur somatic costs, for example, via the risk associated with migration. Migration is usually necessary for the occurrence of outbreeding between individuals of spatially segregated populations of the same species. Migration bears the risk of infection with diseases, lack of familiarity with a local habitat may reduce foraging efficiency, and locally resident conspecifics may attack strangers more severely than familiar individuals (Shields 1993; Pusey and Wolf 1996).

Overall, given the wide range of possible costs and benefits related to different degrees of in- and out-breeding, sexually reproducing organisms should be selected for an optimal balance between in- and out-breeding and achieve the highest fitness at an intermediate genetic distance between mates (Bateson 1983; Shields 1993). Here, we assessed the effects of genetic similarity between mating partners in haplo-diploid plant-inhabiting predatory mites Phytoseiulus persimilis Athias-Henriot (Acari, Phytoseiidae). *Phytoseiulus persimilis* are highly specialized predators of herbivorous spider mites, such as two-spotted spider mites *Tetranychus urticae* Koch, which are worldwide some of the most important crop pests (for review McMurtry and Croft 1997). Spider mites are patchily distributed on their host plants; as a consequence, and also because of mutual attraction (Zhang and Sanderson 1992; Strodl and Schausberger 2012, 2013), also their prime natural enemies, *P. persimilis*, are patchily distributed and live in groups. All phytoseiid species incl. *P. persimilis* are sexually reproducing and haplo-diploid; both females and males arise from fertilized eggs, but males lose the paternal chromosome set during embryogenesis, making them haploid (dubbed pseudo-arrhenotoky; Sabelis and Nagelkerke 1988).

Knowledge of in- and out-breeding is an important issue in *P. persimilis* for its unique sex determination system, its relevance as model species in various branches of science and its importance in pest management. However, despite the obvious relevance for the use of predatory mites in basic and applied research, and practical use in biocontrol, inbreeding has rarely been looked at in predatory mites of the family Phytoseiidae. The only report for *P. persimilis* comes from Poe and Enns (1970), who observed that inbred laboratory colonies of *Neoseiulus fallacis* and *P. persimilis* exhibited high mortality in all life stages and lower viability and reduced fecundity in adult females. In contrast, in the predatory mite *Galendromus occidentalis*, nine generations of sibling mating resulted only in mild inbreeding depression (Hoy 1977). In detail, we investigated the mating behavior of *P. persimilis* at different genetic relatedness levels of mates, the resulting fecundity of females, and the sex ratio of their offspring. Our study aimed at scrutinizing the effects of inbreeding versus outbreeding in a haplo-diploid arthropod and thereby contributing to improve the use of *P. persimilis* in both research and biological control.

Materials and methods

Experimental animals, population origins and rearing

In the experiments, we used individuals of two different laboratory-reared populations of *P. persimilis.* The populations were founded by specimens field-collected in Sicily and Greece, where *P. persimilis* is native (De Moraes et al. 2004), and maintained in the laboratory for about 6–7 years before starting the experiments (Walzer and Schausberger 2011). The laboratory populations had been founded by 50–100 individuals, and population sizes fluctuated between ca. 50 and a few hundred individuals. In the laboratory, the predators were reared on separate artificial arenas consisting of plastic tiles (15 × 15 × 0.2 cm) resting on water-saturated foam cuboids in plastic boxes (20 × 20 × 6 cm) and surrounded by water-saturated tissue paper. The predators were fed by adding leaves of common bean, *Phaseolus vulgaris* L., infested by *T. urticae* onto the arena at 2- to 3-day intervals (McMurtry and Scriven 1965; Schausberger et al. 2016). *Tetranychus urticae* were reared on whole bean plants *P. vulgaris*. Plants were grown at room temperature, 23 ± 2 °C, and artificial light at 16:8 h L:D photoperiod. All rearing and experimental units were kept in a climate chamber at 25 ± 1 °C, 65 ± 5% RH and 16:8 h L:D photoperiod.

Pre-experimental procedures

To obtain, rear and test experimental animals, we used detached bean leaf arenas. Each arena consisted of a detached trifoliate bean leaflet placed upside down on a water-saturated foam cuboid kept in a small plastic box (10 × 10 × 6 cm) half-filled with water. Water-saturated tissue paper covered the edges of the leaf to prevent the mites from escaping.

To obtain even-aged eggs of *P. persimilis*, giving rise to iso-female lines (hereafter called families), gravid females were randomly withdrawn from the rearing units and placed on a detached bean leaf arena infested with *T. urticae*. Predator eggs were collected daily, using a fine marten's hair brush, transferred to new separate leaf arenas infested with *T. urticae*, and reared there until reaching the deutonymphal stage. Deutonymphal females were singly isolated in cylindrical cages of 15 mm diameter and 3 mm height, laser-cut into acrylic plates (75 × 25 mm), closed with fine gauze at the bottom and a microscope slide on the upper side (Schausberger 1997). After reaching adulthood inside the cages, each female was allowed to mate with a male randomly taken from the stock population. Offspring of these couples represented a family and were the individuals used in the experiment. From the egg stage, the offspring of each female (i.e. a family) were split in two groups and reared on two separate arenas, to allow obtaining unfamiliar sibling mates. Two families (iso-female lines) from each population (Sicily and Greece) were used in the experiment.

Experimental procedure

To assess in- and outbreeding depression, we used three levels of genetic relatedness of mates (close, intermediate and distant), i.e. unfamiliar siblings (S), mates from the same population (within population; WP) and mates from different populations (between population; BP). We paired virgin females from a given family with either a male from the same family (S), or a male from another family from the same population (WP), or a male from a family from the other population (BP) (Fig. 1).

The experiment was started by placing one virgin female and one male, randomly taken from its family, into an acrylic cage, which had been previously loaded with surplus spider mites as prey. Subsequently, we checked the cages every 20 min to record the latency until mating occurred and the mating duration for a total of 4–5 h after placing the couples into the cages.

All couples mated successfully and separated again within this time period. Phytoseiid mites mate in the venter-to-venter position, with the male clinging to the female from underneath and transferring the spermatophores with its chelicerae (Amano and Chant 1978). The couples stayed together in their cages for 24 h, with the cages stored upside down on a grid in plastic boxes, the bottom of which was covered by water to elevate the humidity inside the cages (Wimmer et al. 2008). After 24 h, the gravid females were singly transferred onto detached leaf arenas, provided with ample spider mites and left on the arenas until they died of natural causes. Every day we recorded their survival, counted the number of laid eggs, and transferred the eggs onto separate leaf arenas harboring ample spider mites. Eggs collected over a 5-day period were transferred onto the same arena and allowed to grow to adulthood, to determine their sex. Each treatment (S, WP, and BP) was replicated 25–30 times.

Statistical analyses

Statistical analysis was carried out using IBM SPSS 23 (IBM; Armonk, NY, USA). Females not producing a single egg were excluded from analyses, assuming copulation and/or fertilization failure. Such females were rare and equally distributed among the three levels of genetic relatedness (2–4 females per treatment). We compared the time elapsed until mating occurred (mating latency), the duration of mating, the total number of eggs produced by a female and the oviposition period among treatments (level of genetic relatedness of mates) and between populations (Sicily and Greece) by separate generalized linear models (GLM; normal distribution, identity link function). We used full models with two independent main variables, level of mate relatedness and population origin, and their interaction. Least significant difference (LSD) tests were used for post hoc pairwise comparisons between levels of genetic relatedness. The offspring sex ratio was compared among treatments and between populations by GLM (binomial distribution, log-link function).

Results

Neither level of genetic relatedness (GLM; Wald χ_2^2 = 3.037, P = 0.21) nor population (Wald χ_1^2 = 2.475, P = 0.11) nor the interaction between level of relatedness and population (Wald χ_2^2 = 1.679, P = 0.43) had an effect on the mating latency (Fig. 2). The level of genetic relatedness affected the mating duration (GLM; Wald χ_2^2 = 6.305, P = 0.04), which was unaffected by population (Wald χ_1^2 = 0.623, P = 0.73) and the interaction between the level of genetic relatedness and population (Wald χ_2^2 = 4.358, P = 0.11) (Fig. 2): sibling couples (S) mated significantly longer than WP and BP couples (LSD; P ≤ 0.05), while WP and BP couples mated similarly long (P > 0.05).

The level of genetic relatedness significantly affected fecundity (GLM; Wald χ_2^2 = 7.141, P = 0.02) and the oviposition period (Wald χ_2^2 = 5.674, P = 0.05), irrespective of population (Wald χ_1^2 = 0.713, *df* = 2, P = 0.39 and Wald χ_1^2 = 0.744, P = 0.38) and the interaction between level of genetic relatedness and population (Wald χ_2^2 = 0.700, P = 0.42 and Wald χ_2^2 = 0.936, P = 0.62) (Fig. 3). In both populations, WP females produced in total more eggs over a longer time than BP and S females (LSD; P ≤ 0.05 for each pairwise comparison); BP and S females produced similar numbers of eggs and had similarly long oviposition periods (P > 0.05) (Fig.3).

Offspring sex ratio was significantly influenced by both the level of genetic relatedness (GLM; Wald χ_2^2= 17.816, P < 0.001) and population (Wald χ_1^2= 6.515, P = 0.01) as main effects, but not the interaction of level of genetic relatedness and population (Wald χ_2^2= 0.345, P = 0.84). Offspring of females from Greece (mean female proportion ± SE; 0.82 ± 0.01) were more female-biased than offspring of females from Sicily (0.78 ± 0.01). Offspring of WP females (0.76 ± 0.01) were less female-biased than offspring of S (0.81 ± 0.01) and BP (0.83 ± 0.01) females.

Discussion

We assessed excessive in- and outbreeding in *P. persimilis* by comparing the behavioral and reproductive performance of couples having one of three levels of genetic relatedness, that is, mating between siblings, between individuals of the same population and between individuals of different populations. Our experiment revealed a fitness decrease in both closely inbreeding and distantly outbreeding females, suggesting an optimal balance of genetic relatedness of mates belonging to the same population. Sib-mating females and females mating with a male from a different population produced fewer eggs over a shorter time period than females mating with a male from the same population. Mating duration was longer, and the proportion of daughters higher, in sib-mating than within and between population mating couples.

Inbreeding depression is often determined by measuring reproduction after a single generation of inbreeding (Charlesworth and Willis 2009), with many such studies showing analogous results to our study, i.e. inbreeding reducing fecundity (Pusey and Wolf 1996; Saito et al. 2000; Keller and Waller 2002; Swindell and Bouzat 2006; Charlesworth et al. 2007). For pseudo-arrhenotokous predatory mites, Poe and Enns (1970) reported that inbreeding by *P. persimilis* and another predatory mite, *N. fallacis*, resulted in strongly reduced egg production and decreased hatching success of offspring. In contrast, Hoy (1977) observed only mild inbreeding depression in the predatory mite *G. occidentalis*; females from inbred lines had slightly shorter longevity and lower egg production compared to females from outbred lines. In diplo-diploid bulb mites *Rhizoglyphus robini*, Radwan (2003) observed that sib-mating severely reduced fecundity in F1 offspring. In truly arrhenotokous spider mites *T. urticae*, Vala et al. (2003) observed that sib-mating increased mortality, shortened longevity and strongly reduced fecundity in F1 offspring. Similarly, Perrot-Minnot et al. (2004) observed lower fecundity and higher offspring mortality in inbred than outbred *T. urticae*.

Mating latency did not depend on the level of relatedness. However, this may have been due to the no-choice situation and the small size and homogeneity of the experimental cages, promoting mate encounter and generally shortening the latencies, as compared to leaves. On leaf arenas, which are more heterogeneous and where mate finding is more difficult, mating latency has been shown being an appropriate indicator of mate choice. For example, Enigl and

Schausberger (2004) observed that virgin *P. persimilis* females tended to accept unrelated mates earlier than related mates. The mean mating duration exceeded in all three treatments (level of genetic relatedness of couples) of both populations the species-specific threshold needed for complete successful insemination, which is about 80 min at 25 °C (Walzer and Schausberger 2015). Below this threshold, the mating duration is positively linked to the volume of the endo-spermatophores following spermathecal insemination (Amano and Chant 1978; Schulten 1985). The significantly longer mating duration in sib-mating couples than in within- and between-population couples thus likely reflects decreased behavioral compatibility of sib couples, for example, because of female reluctance to allow unrestricted filling of both spermathecae, and thus suboptimal spermatophore transfer by the males (Schausberger et al. 2017).

The offspring sex ratio of predatory mites is mostly female-biased, usually at around two females to one male, under favorable conditions (Hoy 1977; Dyer and Swift 1979; Walter 1992). In many animal taxa, the offspring sex ratio is influenced by the level of inbreeding, with a positive correlation between the level of inbreeding and the male bias among offspring (Greeff and Taylor 1997; Frankham and Wilcken 2006). However, in view of kin selection theory, to reduce local mate competition among sons (Hamilton 1967), females mating with a close relative should reduce the male bias and close inbreeding should hence result in more female-biased offspring sex ratios (West et al. 2002). In haplo-diploid species, only daughters will be affected by homozygosity problems arising from inbreeding while sons will be unaffected (Antolin 1999; Henter 2003). Hoy (1977) reported that *G. occidentalis* showed only mild inbreeding depression after nine generations without any alterations of offspring sex ratio. In contrast, in our study both sib-mating females and between-population mating females shifted the offspring sex ratio towards daughters. Reducing local mate competition as ultimate explanation is only applicable to sib-mating females; an alternative or additional explanation, applying to both sib-mating and between-population mating females, is mothers counterbalancing the fitness decrease of individual daughters, accruing from reduced fecundity, by increasing the proportion of daughters among offspring.

In summary, our findings support the hypothesis of optimal outbreeding (Bateson 1983). Both excessive inbreeding and distant outbreeding reduced the fitness of *P. persimilis*, relative to an intermediate level of mate relatedness. Nonetheless, our study only quantified the effects of mate relatedness in the F_1 generation. To gain a more comprehensive understanding, follow-up studies should scrutinize long-term trans-generational consequences of founder relatedness effects and chronic inbreeding on population growth and persistence. Taken together, our outcomes should foster the use of *P. persimilis* as model species in studies on in- and out-breeding by haplo-diploid organisms (Henter 2003). The outcomes should contribute to improve the use of *P. persimilis* in both fundamental and applied research, for example, by optimizing laboratory and commercial rearing, and allow tailoring efficient release and establishment strategies in augmentative and classical biocontrol.

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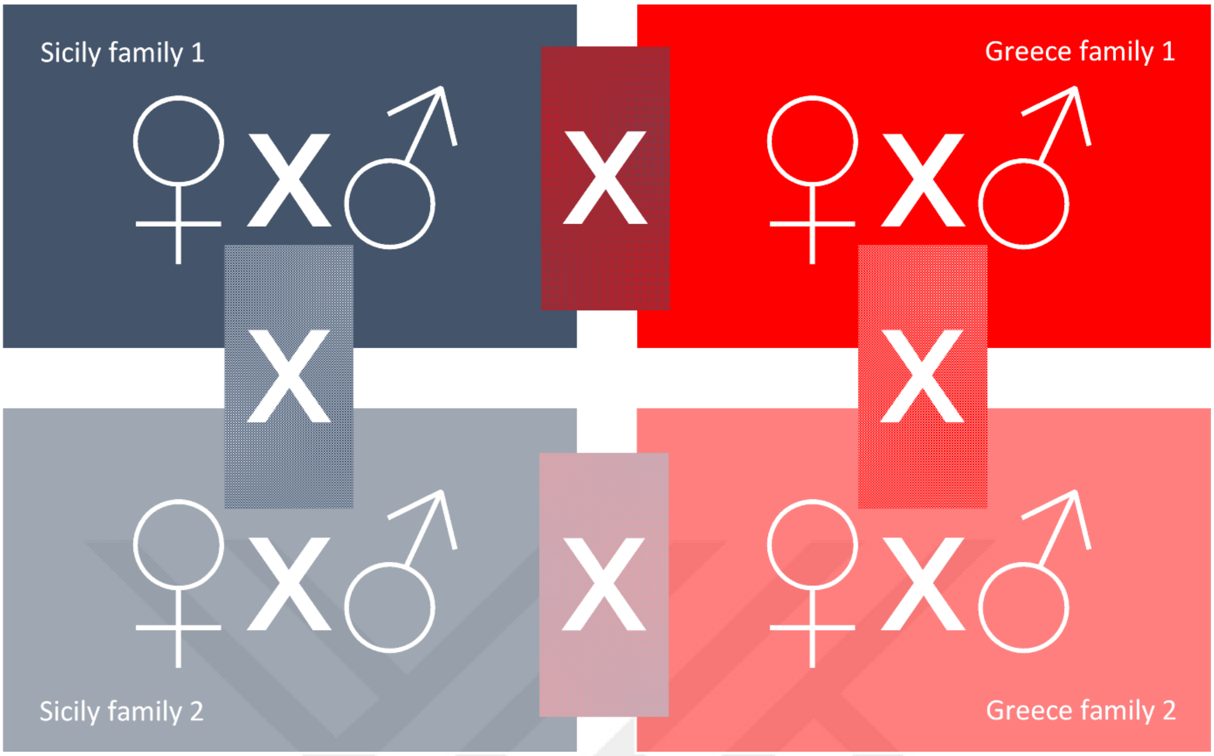
Figure captions

Fig. 1 Crossing design used to assess in- and out-breeding depression. Families were iso-female lines established from populations originating from Sicily and Greece. Mates came either from the same family (sibling mating), or from different families from the same population (within population; WP), or from different families from different populations (between population; BP).

Fig. 2 Mating duration and mating latency. Mating partners came either from the same family (siblings), or from different families from the same population, or from different families from different populations (Sicily and Greece).

Fig. 3 Total number of eggs and oviposition period. Mating partners came either from the same family (siblings), or from different families from the same population, or from different families from different populations (Sicily and Greece).



Sicily family 1
Greece family 1
Sicily family 2
Greece family 2

Fig. 1

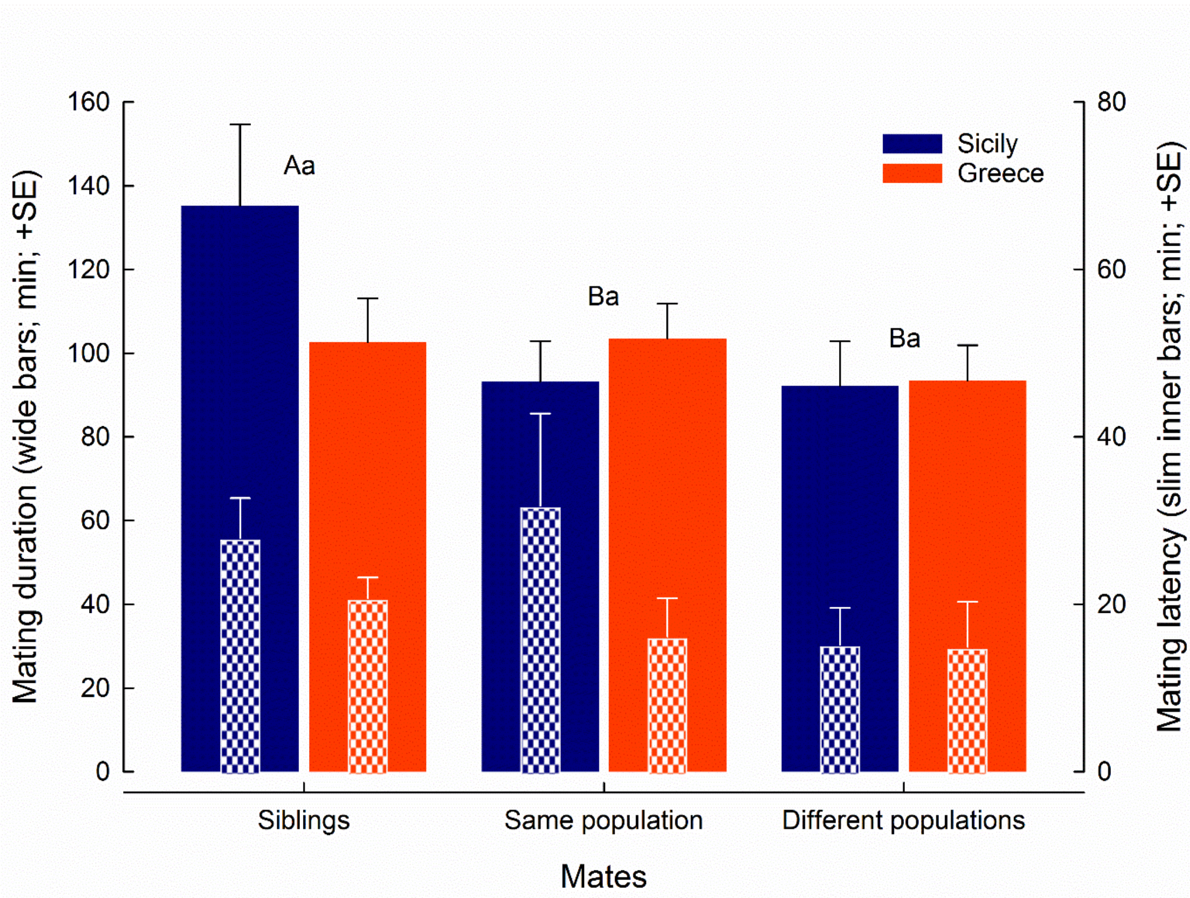
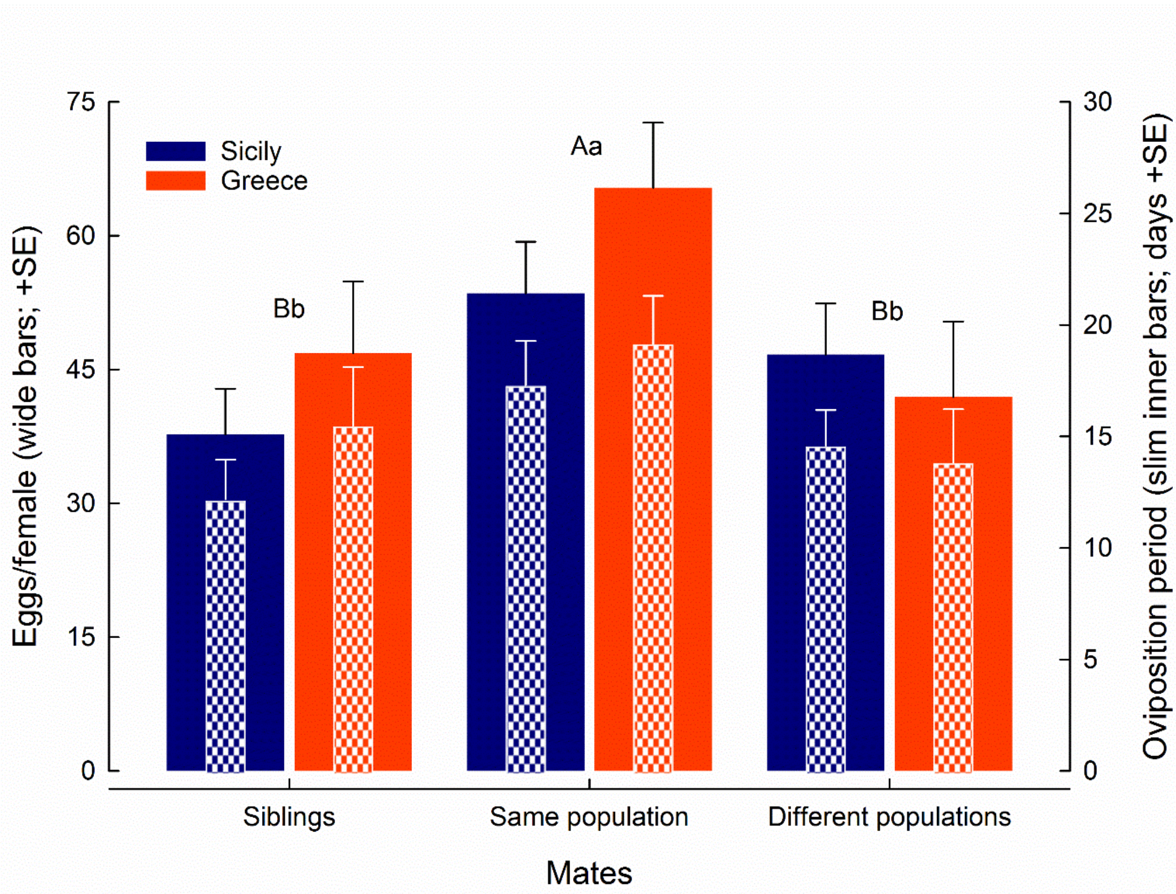

Mating duration (wide bars; min; +SE)
160
140
120
100
80
60
40
20
0
Aa
Ba
Ba
Sicily
Greece
80
60
40
20
0
Mating latency (slim inner bars; min; +SE)
Siblings
Same population
Different populations
Mates

Fig. 2



Sicily
Greece
Aa
Bb
Bb
Eggs/female (wide bars; +SE)
Oviposition period (slim inner bars; days +SE)
75
60
45
30
15
0
30
25
20
15
10
5
0
Siblings
Same population
Different populations
Mates

Fig. 3

2.2

Founder effects on trans-generational dynamics of chronically inbreeding lineages of the predatory mite *Phytoseiulus persimilis*

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Abstract

Both close inbreeding and distant outbreeding may reduce fitness below the level of individuals with intermediate parental relatedness. In the haplodiploid plant-inhabiting predatory mite *Phytoseiulus persimilis*, which is patchily distributed within and among host plants, fitness is indeed reduced in the short term, i.e. by a single generation of inbreeding. However, in the medium to long term (multiple generations), distant out-breeding should provide for favorable demographic founder effects in isolated populations. We tested this prediction in isolated experimental lineages founded by females mated to a sibling (close inbreeding), a male from the same population (intermediate relatedness) or a male from another population (distant outbreeding) and monitored lineage growth and persistence over four generations. Cross-generationally, lineages founded by distantly outbred females performed the best, i.e. produced the most descendants. However, this was solely due to superior performance from the F_2 generation onwards, whereas in the F_1 generation, lineages founded by females mated to males from their own population (intermediate relatedness) performed the best, as predicted from short-term in- and out-breeding depression effects. At the genetic level, this result was most likely due to distantly outbred founders introducing higher allelic variability and lower homozygosity levels, counterbalancing inbreeding depression, which inevitably occurs in isolated lineages, from the F_2 generation onwards.

Introduction

Founder effects, i.e. effects emerging from founding of populations/lineages by a subset of individuals of the parental population, have decisive influence on the occurrence and intensity of inbreeding depression [1,2]. Founder effects change the genetic composition, usually by reducing variation, of daughter populations/lineages relative to the parental population and are thus especially relevant for inbreeding by organisms structured in small and fragmented or locally isolated populations with limited genetic exchange [3,4].

The detrimental effects of close inbreeding were already studied and documented by Darwin (1876) [5] for various plant species. At the genetic level, increased homozygosity commonly results in fitness decrease, called inbreeding depression, with overdominance (general advantage of heterozygotes) or partial dominance (homozygosity of recessive deleterious alleles) as the underlying mechanisms [6–8]. At the species level, averaged across individuals and assuming partial dominance as causal mechanism, the deleterious effects of inbreeding depression are expected to be lower in haplodiploids than in diplodiploids. The reason is that in haplodiploid organisms with hemizygous males, recessive deleterious alleles should be more easily and more quickly purged than in diplodiploid organisms with heterozygous males [9–12]. Nonetheless, while at large inbreeding depression is indeed less severe in haplodiploid than in diplodiploid organisms [13–15], excessive inbreeding may cause significant negative fitness effects in haplodiploids as well [10,16–18]. Towards the distant end of the mate relatedness continuum, also excessive outbreeding may cause significant negative effects [19–21]. Common underlying reasons are behavioral incompatibilities at the phenotypic level, and genetic incompatibilities, break-down of co-adapted gene complexes and maladaptation at the genotypic level [7,22–23]. Therefore, according to optimal outbreeding theory [19], intermediate levels of mate relatedness should provide for the highest fitness [22,24].

Nonetheless, regarding the effects of mate relatedness on in- and out-breeding depression, the time scale and associated number of generations, i.e. distinction between short- (one generation) and medium- to long-term (multiple generations) effects, are decisive variables. Short-term uni-generational effects of mate relatedness on in- and out-breeding depression may differ from medium- to long-term trans-generational effects. The effects of chronic inbreeding, which inevitably occurs in isolated lineages founded by single or few individuals, because of increasing mate relatedness over time, should heavily depend on the genetic composition and diversity introduced by the founder individuals, i.e. their in- and outbreeding status [25–27]. Outbred founders should provide for increased genetic variability and/or decreased homozygosity, favoring trans-generational lineage growth and persistence. Under these circumstances, distant outbreeding by founders may be sub-optimal in the short term but may be more favorable than close and intermediate founder mate relatedness levels in the medium and long run [26,28].

We addressed this issue, that is, trans-generational effects of founder relatedness, in isolated experimental lineages of plant-inhabiting predatory mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). *Phytoseiulus persimilis* are haplodiploid, with both females and males arising from fertilized eggs but males losing the paternal chromosome set during embryogenesis, a phenomenon dubbed pseudo-arrhenotoky [29]. *Phytoseiulus persimilis* are specialized predators of herbivorous spider mites, which are patchily distributed both at the local (within leaves and plants) and regional (among plants) spatial scale. Due to the spider mites' patchy distribution pattern and mutual attraction, also their predators, *P. persimilis*, are patchily distributed within and among plants [30,31]. Local dispersal by *P. persimilis* occurs by ambulation whereas long-distance dispersal is passive using the wind [32]. *Phytoseiulus persimilis*' prey specialization level, patchy distribution pattern and dispersal modes set the stage for the occurrence of in- and out-breeding and founder effects. However, knowledge of the consequences of in- and out-breeding by *P. persimilis* is very limited [33,34] and experimental studies addressing founder effects are lacking. *Phytoseiulus persimilis* has a short developmental time (5 to 6 d at 25˚C) and high fecundity (up to 5 eggs/d at 25˚C), resulting in intrinsic rates of increase (r_m) >0.3/d [35,36]. Its specialized feeding habits and high population growth capacity render *P. persimilis* a highly efficacious, globally used biocontrol agent of spider mites [36]. Knowing the consequences of in- and out-breeding is imperative for optimizing *P. persimilis*' mass-rearing and release strategies.

Recently, we scrutinized short-term (one generation) individual fitness effects of mate relatedness in *P. persimilis* [34]. In accordance with optimal outbreeding [19], we observed that an intermediate level of mate relatedness (same population) resulted in higher female fitness (higher fecundity) than excessively close (siblings) or distant (from geographically widely separated populations) mate relatedness [34]. The present study aimed at elucidating demographic founder effects, at graded levels of genetic relatedness between the founding female and her mate (sibling, same population and different population), in experimental lineages derived from two geographically widely separated populations of *P. persimilis*, Sicily and Greece. We hypothesized that chronically inbreeding lineages founded by outbred females, i.e. females mated to males from another population, produce more descendants and persist at higher abundance levels than lineages founded by sib-mated females and lineages founded by females mated to males from their own population. To experimentally increase the likelihood of occurrence and detectability of founder effects on inbreeding depression of *P. persimilis*, all lineages were subjected to food stress, i.e. held under limited prey conditions over 40 days, allowing about four generations (generation time [T] is ~10 to 11 d at 25˚C) [36,37]. Under stressful conditions, in the short to medium term, recessive alleles are commonly more detrimental, and increase inbreeding depression effects, compared to benign conditions [26,38,39]. In the long term, chronically prevailing stress conditions may promote purging of deleterious recessive alleles and thereby lead to reduced inbreeding depression, as for example shown for fruit flies, *Drosophila melanogaster* [40,41].

Materials and methods

Experimental animals, population origins and rearing

Experimental animals derived from iso-female lines established from two laboratory-reared populations of *P. persimilis*. One population was founded by specimens collected in Sicily and the other by specimens collected in Greece [42]. *Phytoseiulus persimilis* is native at both sampling locations [43]; wide geographic separation suggests minimal genetic exchange, if occurring at all, between *P. persimilis* from Sicily and Greece. Each population was founded by 50 to 100 individuals and population sizes fluctuated between ~50 and a few hundred individuals. Both populations were maintained in the laboratory for about 6 to 7 years before starting the experiment.

In the laboratory, the predators were reared on separate artificial arenas consisting of plastic tiles (15 x 15 x 0.2 cm), delimited by water-saturated tissue paper, and resting on water-saturated foam cubes in plastic boxes (20 x 20 x 6 cm) half-filled with water. The predators were fed by adding leaves of common bean *Phaseolus vulgaris* L., infested by *Tetranychus urticae* Koch, onto the arena at two to three-day intervals [44,45]. *Tetranychus urticae* was reared on whole common bean plants *P. vulgaris*. For the experiments, the spider mites were brushed from infested leaves, using a paint brush or a mite brushing machine (BioQuip, CA, USA), onto glass plates, and then either singly picked up and placed into acrylic cages using a fine red marten's hair brush or directly brushed onto detached leaf arenas or artificial tile arenas. Plants were grown at room temperature 23 ± 2˚C and 16:8 h L:D photoperiod. All rearing and experimental units were kept in a climate chamber at 25 ± 1˚C, 65 ± 5% RH and 16:8 h L:D photoperiod.

Establishing iso-female lines

Founder females of the experimental lineages came from iso-female lines of *P. persimilis*, which were established as described by Atalay & Schausberger (2018) [34]. To establish isofemale lines, gravid females were randomly withdrawn from the stock populations and placed on spider mite-infested detached bean leaf arenas, each consisting of a trifoliate leaf placed upside down on a water-saturated foam cuboid, kept in a small plastic box (10 x 10 x 6 cm) half-filled with water, and delimited by water-saturated tissue paper. Eggs laid by the predators were transferred to new *T. urticae*-infested arenas and reared there until the deutonymphal stage. Deutonymphal females were singly isolated in cylindrical acrylic cages of 15 mm diameter and 3 mm height, laser-cut into rectangular acrylic plates (7.5 x 2.5 x 0.3

cm), closed with fine gauze at the bottom and a microscope slide on the upper side [46]. After reaching adulthood, each female was mated with a male randomly withdrawn from the stock population. Offspring of these couples represented a family (iso-female line) and were the individuals used in the experiment. Two families from each population (Sicily and Greece) were used in the experiment (Fig 1). Using the family design allowed comparing in- and out-breeding by individuals having the same set of alleles [39]. According to Nelson & Greeff (2011) [47], the inbreeding coefficient (f_t) [48] for offspring of full sib-mates (0.25) is almost equal in haplodiploids and diplodiploids [49]. In our experiment, we assumed inbreeding coefficients (*f1*) of 0 for offspring of distantly outbreeding couples (different populations), 0.125 for offspring of couples of the same population and 0.275 for offspring of sib couples. The latter two coefficients were based on the assumption of parental f_0 = 0.1 because founders were withdrawn from closed and thus inbred laboratory populations [50]. The predicted increase in inbreeding coefficients of the three types of experimental lineages (Fig 2) was estimated with *Populus* 5.5 [51].

Experimental procedure

The experiment was started by placing one virgin female and one male, at each level of genetic relatedness (siblings, same population, other population) from both populations, in acrylic cages, previously loaded with mixed spider mite stages, for 24 h (Fig 1). Cages were stored upside down on a grid in plastic boxes, the bottom of which was covered by water to warrant elevated humidity inside the cages [52]. After 24 h, the now mated females were singly placed on an acrylic arena, consisting of a plastic tile (15 x 15 x 0.2 cm) delimited by water-saturated tissue paper and resting on a water-saturated foam cuboid inside a plastic box (20 x 20 x 6 cm) half-filled with water. The predators were provided with predefined standardized amounts of mixed spider mite stages (i.e., brushed from three similarly sized bean leaves, each infested by ~50 to 80 *T. urticae*) in 3 d intervals. Over 40 days (allowing approximately four predator generations), we recorded the number and life stages of the predators (eggs, juveniles, adult females and adult males) in 5 d intervals. Each of the three levels of founder mate relatedness was replicated 13 to 22 times.

Statistical analysis

Statistical analysis was carried out using IBM SPSS 23 (IBM; Armonk, NY, USA). For analysis, we used only counts of mobile predator life stages since accurately counting predator eggs was difficult on the arenas containing live prey and their webbing, and prey corpses. We used separate generalized estimating equations (GEE [53]; normal distribution, identity link function; M-dependent correlation matrix between observation days) to compare the influence of founder inbreeding level and population origin on lineage dynamics (abundance of mobile individuals) over the whole experimental period (40 d) and split into the first generation (<15 d) and from the onset of the second generation onwards (>10 d). Model selection was based on the QIC (quasi likelihood under independence model criterion) value. Mean generation time of *P*. *persimilis* is ~10 to 11 d at 25 ˚C [36,37,54]. In case of a significant interaction term between founder inbreeding level and population origin, we conducted additional GEEs for the influence of mate relatedness on lineage dynamics within each population origin (Sicily and Greece). For post-hoc pairwise comparisons between founder inbreeding levels, we used least significant difference tests (LSDs).

Results

Mean abundance of predator lineages over the whole experimental period was significantly influenced by the interaction between founder inbreeding level and population origin (GEE: *Wald* χ_2^2 = 6.402, *P* = 0.04), whereas neither founder inbreeding level (*Wald* χ_2^2 = 2.749, *P* = 0.25) nor population origin (*Wald* χ_1^2 = 0.281, *P* = 0.59) exerted significant main effects (Fig 3). Within either population origin, mean lineage abundance varied significantly with founder inbreeding level (GEE for Sicily: *Wald* χ_2^2 = 7.683, *P* = 0.02; Greece: *Wald* χ_2^2 = 6.319, *P* = 0.04). For the Sicily origin, lineages founded by sib-mated females reached significantly lower abundances (on average 20 to 30% lower) than lineages founded by females mated to males from the same population or to males from Greece (LSD: $P < 0.05$) (Fig 3). For the Greece origin, lineages founded by females mated to males from Sicily reached significantly higher abundances (on average 20% higher) than the population founded by females mated to males from the same population (LSD: $P = 0.01$) (Fig 3). All other pairwise comparisons were non-significant ($P > 0.05$). Two lineages founded by sib-mated females (one from Sicily and one from Greece) went extinct within the F_1 generation (no individuals observed on day 15 and 20, respectively). All lineages founded by females mated to males from the same or the other population persisted until the end of the experiment.

Mean lineage abundance of the F_1 generation (until day 10) was significantly influenced by founder inbreeding level (GEE: $Wald\ \chi_2^2 = 8.878$, $P = 0.01$) and marginally by founder population origin ($Wald\ \chi_1^2 = 3.028$, $P = 0.08$) but not by the interaction of founder inbreeding level and population origin ($Wald\ \chi_2^2 = 1.979$, $P = 0.37$) (Fig 4). For both origins, Sicily and Greece, lineages founded by females mated to males from the same population reached significantly higher abundances (on average 30 to 35% higher) than lineages founded by sib-mated females (LSD: $P = 0.007$) and lineages founded by females mated to males from the other population ($P = 0.01$).

Mean lineage abundance from day 15 onwards (F_2 to F_4), i.e. after the onset of the F2 generation, was significantly influenced by founder inbreeding level (GEE: $Wald\ \chi_2^2 = 6.602$, $P = 0.03$) but not by founder population origin ($Wald\ \chi_1^2 = 1.535$, $P = 0.21$) and the interaction of founder inbreeding level and population origin ($Wald\ \chi_2^2 = 4.121$, $P = 0.12$) (Fig 4). For both origins, Sicily and Greece, lineages founded by females mated to males from the other population reached significantly higher abundances (on average 15 to 20% higher) than lineages founded by sib-mated females (LSD: $P = 0.02$) and lineages founded by females mated to males from the same population ($P = 0.05$).

Discussion

Our study documents demographic founder effects in dependence of the founders' in- and out-breeding status in the haplodiploid plant-inhabiting predatory mite *P. persimilis*. Trans-generationally, that is, over approximately four generations, closed lineages founded by distantly outbred females (mates coming from different populations) reached higher abundance levels than lineages founded by heavily (sibling mates) and lightly (mates coming from the same population) inbred females. However, time-dependent inter-lineage comparison revealed superior performance of lineages founded by outbred females only from the F_2 generation onwards, whereas in the F_1 generation lineages founded by females with intermediate mate relatedness levels (same population) performed better than lineages founded by sibmated females and lineages founded by distantly outbred females (different populations).

Superior performance of lineages founded by females with intermediate mate relatedness in the F_1 generation conforms to the predictions of optimal outbreeding theory [19] and was expected from beneficial short-term (F_1 generation) individual fitness effects [34]. In the short term, females mated to males from the same population have a higher fecundity and longer oviposition period than females mated to siblings and males from another population [34]. Accordingly, in this study, lineages founded by females mated to males from the same population reached higher abundances in the F_1 generation than lineages founded by sib-mated females and females mated to a male from the other population.

Throughout the experiment, all lineages were provided with the same limited amount of prey, hence food stress increased with increasing predator abundance in the course of the experiment. Limited food availability is a prime stressor during population development, fostering intraspecific competition and increasing individual starvation, retarding development, decreasing reproduction, and promoting propensities to dispersal and cannibalism. Higher frequencies of deleterious recessive alleles and homozygosity make inbred individuals commonly more sensitive to stressful conditions than outbred individuals [39]. Food stress was negligible in the F_1 generation but increased from the F_2 generation onwards. Beginning with the F_2 generation, growth and abundance of sib-mated lineages and within population lineages was similar yet lower than growth and abundance of between population lineages. Obviously, within population lineages exceeded a critical inbreeding coefficient in the F_2 generation (Fig 2), beyond which inbreeding depression effects became similar to those of lineages founded by sib-mated females. At the individual phenotypic level, genetic deficiencies associated with increasing inbreeding levels, may have adversely affected diverse inter-related physiological, life history and/or behavioral traits, such as decreasing general stress resilience, decreasing tolerance of energetic constraints, increasing mortality, prolonging development, decreasing fecundity, decreasing competitive abilities, and promoting the occurrence of cannibalism and dispersal tendencies [27,55,56,57]. Pinpointing the phenotypic mechanisms underlying the differences in performance of isolated lineages emerging from in- and out-bred founders requires additional experimental work.

Inter-lineage comparison from the F_2 generation onwards revealed superior performance, that is, higher population growth and abundance, of lineages founded by outbred females, most likely because of higher allelic variability and lower homozygosity frequency alleviating inbreeding depression effects. Similarly, in the seminal experiments by Ayala (1965) [25], isolated populations of fruit flies *Drosophila serrata* emerging from genetically diverse founders reached higher growth rates and abundances than populations founded by genetically similar individuals. Saccheri et al. (1998) [58] identified increasing extinction risk with decreasing heterozygosity in natural populations of the Glanville fritillary butterfly *Melitaea cinxia*. Hildner, Soule, Min, & Foran (2003) [59] found an association between higher genetic variability and higher population growth rates in pocket gophers, *Thomomys bottae*. Agashe (2009) [60] and Agashe, Falk, & Bolnick (2011) [61] observed that populations of the red flour beetle *Tribolium* *castaneum*, founded by genetically diverse individuals, performed better (decreased extinction risk, faster adaptation in novel environment) than populations founded by genetically similar individuals. Apart from long-term outbred founder benefits, because of higher allelic diversity, outbreeding may also exert immediate positive fitness effects on population growth by heterosis, increasing hybrid vigor in the F_1 generation [7,26,38]. For example, Drake (2006) [62] suggested heterosis being the prime genetic mechanism boosting population growth (dubbed "catapult effect"), and thus promoting establishment success in the wild, of red-necked pheasants *Phasianus colchicus*. Similarly, Wagner, Ochocki, Crawford, Compagnoni, & Miller (2017) [63] suggested heterosis causing a catapult effect in experimental lineages of the weevil *Callosobruchus maculatus*. However, in our study on *P. persimilis*, heterosis did not play a role because in the F_1 generation lineages of outbred founders did worse than lineages of founders with intermediate level of relatedness.

Overall, our findings reveal that founder relatedness decisively influences the dynamics of chronically inbreeding lineages of the predatory mite *P. persimilis*. Our findings are highly relevant for the use of *P. persimilis* and other pseudo-arrhenotokous predatory mite species in augmentative and classical biological control of herbivorous mites, aiming at medium- to long-term predator establishment in novel environments. Our study predicts that colonization, establishment success and population growth and persistence of *P. persimilis* and other predatory mites should be higher when releasing outbred founders [64]. Answering for how long such founder effects will last and whether purging allows populations founded by inbred founders to catch up requires further investigations.

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Figure captions

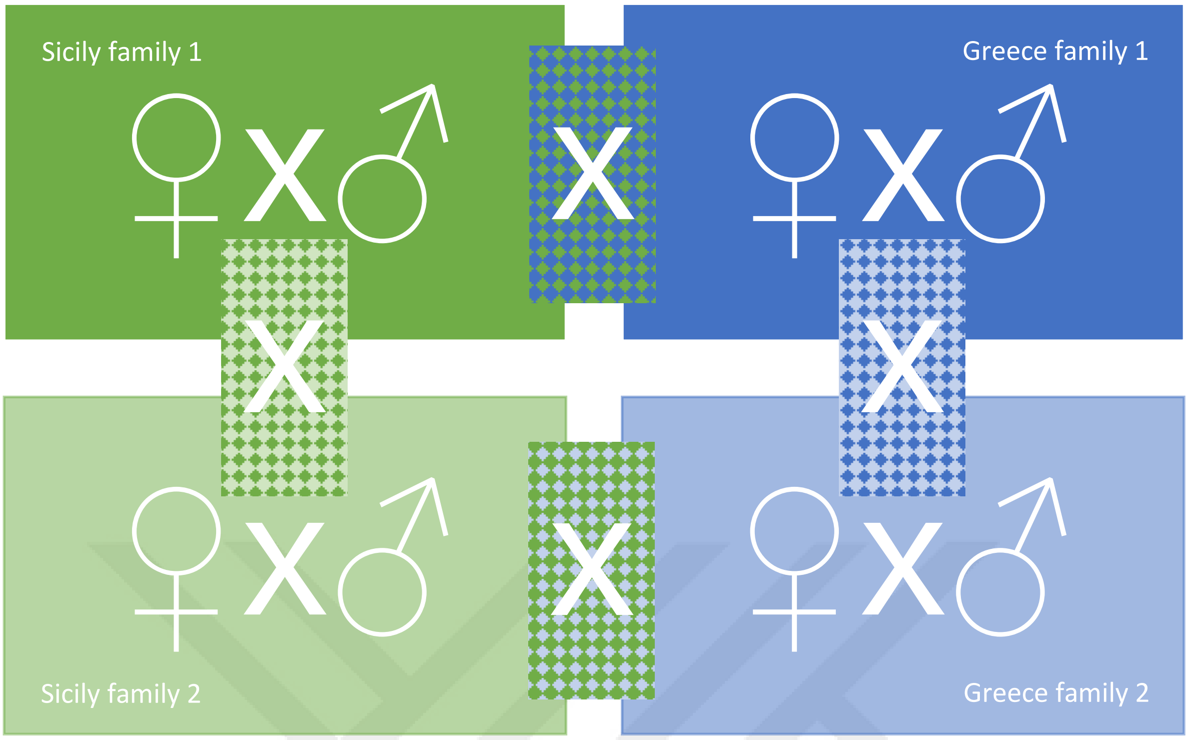
Figure 1. Crossing design used to establish three levels of in- and outbreeding in the founder females of the experimental lineages of *P*. *persimilis* (original figure [34]). Families were iso-female lines established from populations originating from Sicily and Greece. Mates came either from the same family (siblings), or from different families of the same population, or from different families from different populations.

Figure 2. Predicted trans-generational development of Wright's (1922) [48] inbreeding coefficient (f_t) in chronically inbreeding experimental lineages of *P*. *persimilis* founded by females mated to a brother, a male from the same population or a male from another population (curve estimation using *Populus* 5.5 [51]). Assumed female to male inbreeding coefficients (f_0) of the parental generation (F_0) were 0.1 for sibling and same population mates and 0 for different population mates. Accordingly, f_1 was 0, 0.125 and 0.275 for offspring (F_1) of founders mated to males from different populations, the same population and siblings, respectively [50].

Fig 3. Trans-generational dynamics of experimental lineages founded by single *P*. *persimilis* females from Sicily and Greece. Founding females were mated to males with graded levels of genetic relatedness (siblings, same population, different populations) and lineage abundance (number of mobile mites) recorded over 40 days, corresponding to approximately four generations.

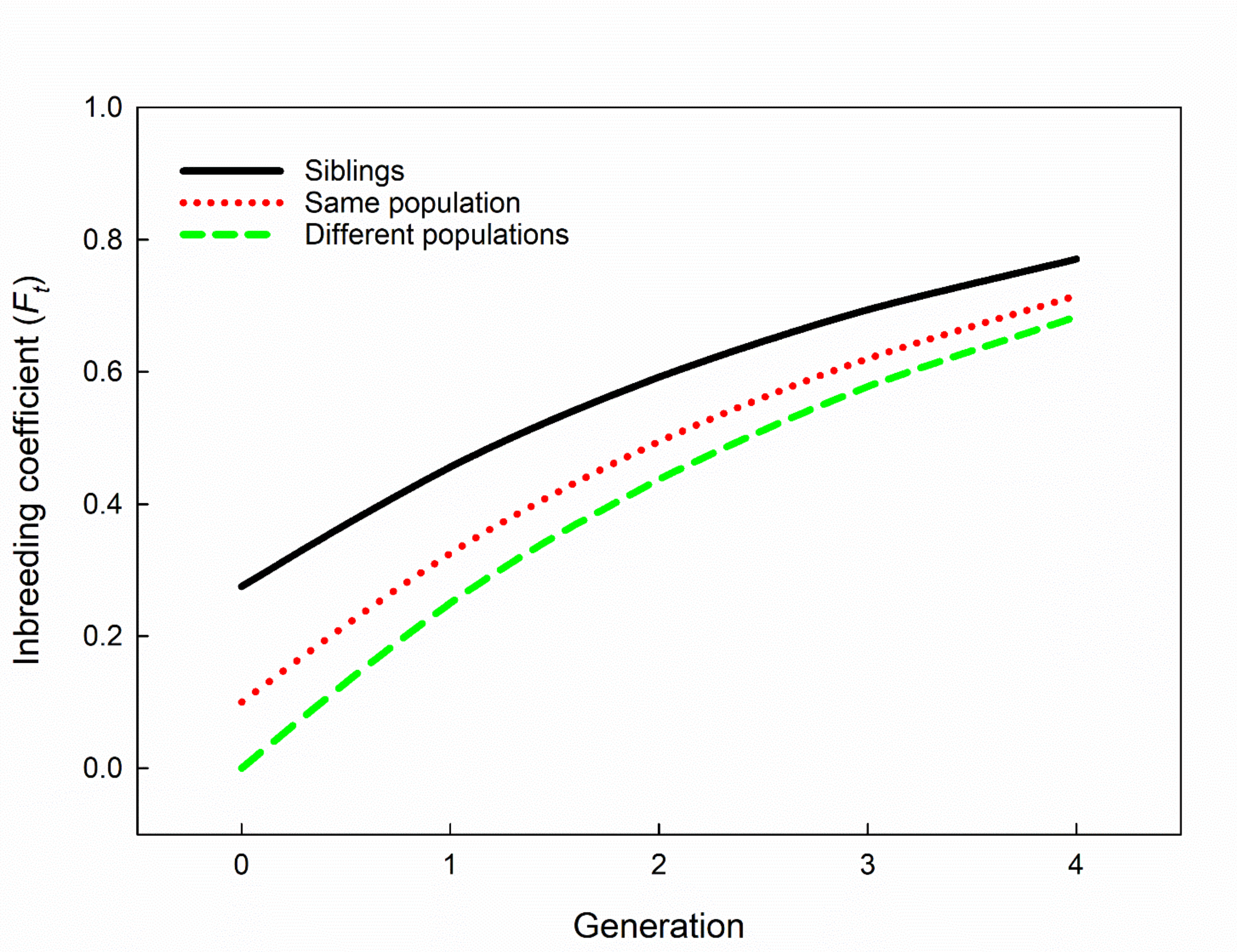
Fig 4. Mean generation-specific abundance of experimental lineages of *P*. *persimilis* founded by single females from Sicily or Greece, mated to males with graded levels of genetic relatedness (siblings, same population, different populations). Lineage abundance (number of mobile mites) was separately analyzed on days 5 and 10 of the experimental period (F_1 generation; before F_1 females started reproduction) and from day 15 onwards (F_2 to F_4 generations; after F_1 females started reproduction). *P*-values refer to pairwise comparisons (LSD) between founder relatedness levels following GEE.

Fig. 1



Sicily family 1
♀ X ♂
X
Greece family 1
♀ X ♂
X
X
♀ X ♂
X
♀ X ♂
Sicily family 2
Greece family 2

Fig. 2



1.0
0.8
0.6
0.4
0.2
0.0
Inbreeding coefficient (F_t)
Siblings
Same population
Different populations
0
1
2
3
4
Generation

Fig. 3

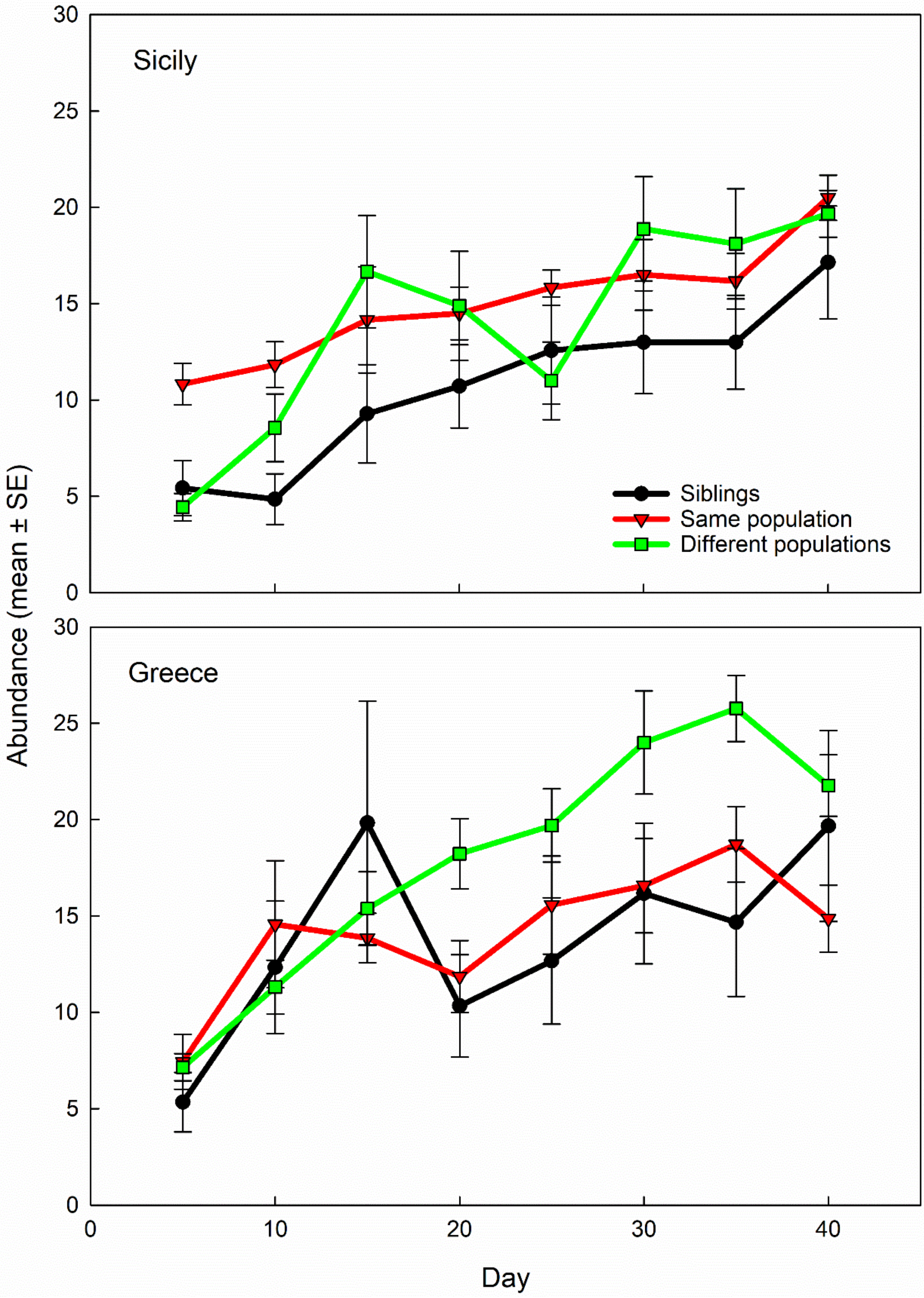
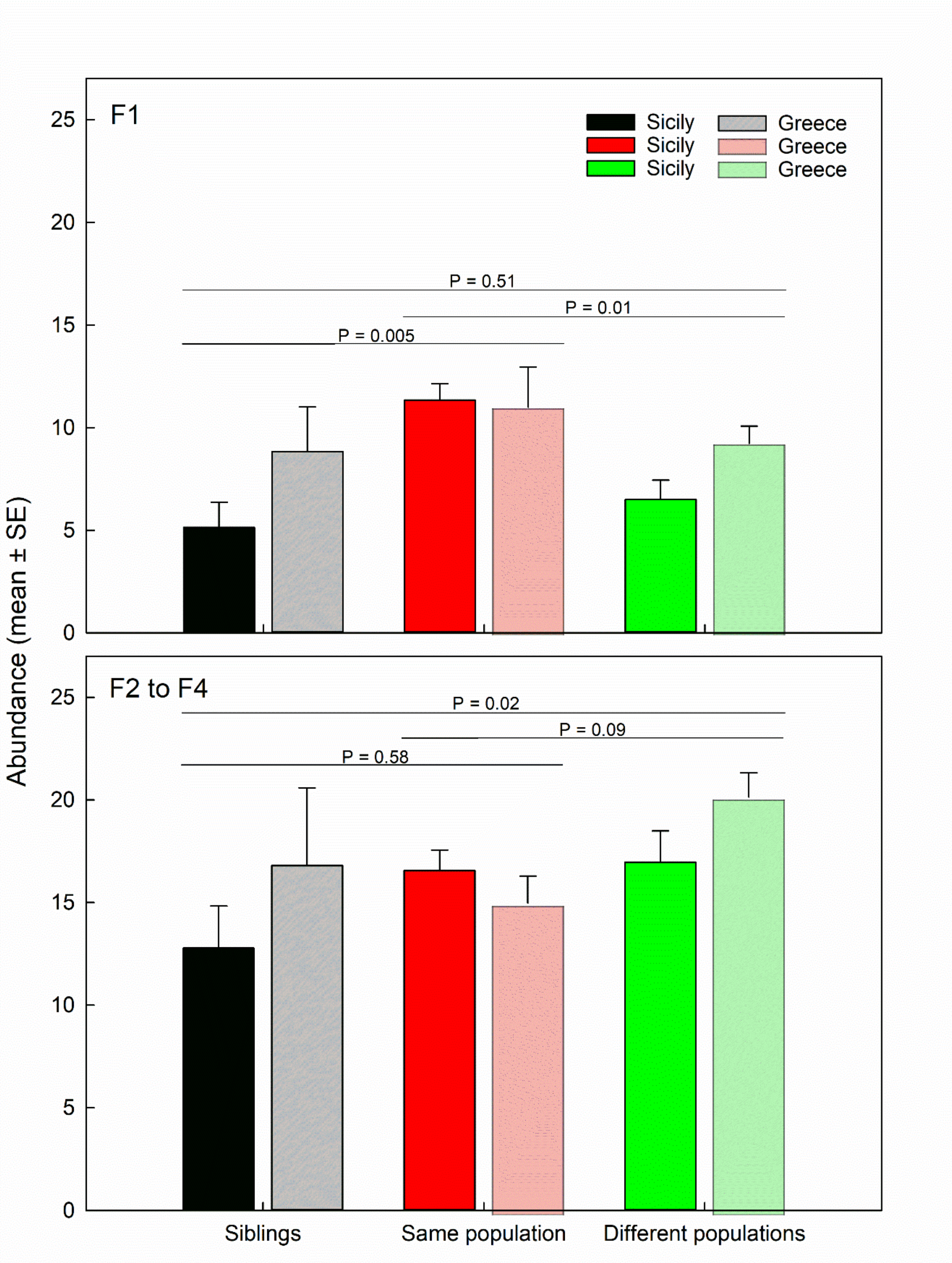

Sicily
Greece
Siblings
Same population
Different populations
Abundance (mean ± SE)
Day
30
25
20
15
10
5
0
0
10
20
30
40

Fig. 4



F1
Sicily
Sicily
Sicily
Greece
Greece
Greece
P = 0.51
P = 0.01
P = 0.005
F2 to F4
P = 0.02
P = 0.09
P = 0.58
Abundance (mean ± SE)
0
5
10
15
20
25
Siblings
Same population
Different populations

2.3

Plastic female choice to optimally balance (k)in- and out-breeding in a predatory mite

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Abstract

Both close inbreeding and distant outbreeding may negatively affect direct fitness. Optimal outbreeding theory suggests that, to maximize fitness, females should preferentially mate with distantly related males. Optimal (k)in breeding theory suggests that, at similar direct fitness costs of close inbreeding and distant outbreeding, females should prefer close kin to non-kin to secure indirect fitness benefits. While there exists ample evidence of female choice to avoid close inbreeding, empirical evidence of flexible female choice for an optimal balance between close inbreeding and distant outbreeding remains elusive. Here we tested the combined predictions of optimal outbreeding and kin breeding theories in plant-inhabiting predatory mites *Phytoseiulus persimilis* from two origins, Sicily and Greece, which suffer from both close inbreeding and distant outbreeding depression. Virgin females were presented binary choices between brothers and distant kin (same population) or non-kin (other population). To unveil possible kin recognition mechanisms, brothers were either familiar or unfamiliar to the female. Females of Greece but not Sicily preferred unfamiliar brothers to familiar brothers. Females of both origins preferred distant kin to unfamiliar and familiar brothers but preferred unfamiliar brothers to non-kin. Females of Sicily but not Greece preferred familiar brothers to non-kin. The suggested kin recognition mechanisms are phenotype matching and/or direct familiarity, with finer-tuned recognition abilities in the Greece population. Taken together, our experiments suggest that females of *P. persimilis* are able to flexibly adjust mate choice between close kin, distant kin and non-kin to optimally balance direct and indirect fitness benefit-cost trade-offs.

Keywords Inbreeding, kin recognition, female choice, mites, optimal balance, outbreeding

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1. Introduction

Both close inbreeding and distant outbreeding may negatively affect fitness. Close inbreeding commonly decreases direct fitness by, for example, reducing survival, reproduction, competitiveness and/or stress tolerance (e.g. Wright 1977; Charlesworth and Charlesworth 1987; Fox and Reed 2011). However, due to increased genetic relatedness of progeny, inbreeding entails indirect fitness gains (Hamilton 1964ab), counterbalancing or even outweighing direct fitness costs (Taylor and Gets 1994; Kokko and Ots 2006; Puurtinen 2011). Outbreeding is often advantageous to close inbreeding but the benefit-cost trade-offs depend on the level of genetic distance between mates and the choice options. Extreme outbreeding nullifies indirect fitness gains but may also be detrimental to direct fitness for various reasons such as underdominance, breaking up advantageous co-adapted gene complexes or maladaptation (Bateson 1983, Templeton 1986, Thornhill 1993, Peer and Taborsky 2005). Accordingly, synthesis of optimal outbreeding theory (Bateson 1983) and optimal (k)in breeding theory (Puurtinen 2011) suggests that animals should optimally balance mate choice between kin and non-kin, in dependence of the choice options and the associated benefit-cost trade-offs in direct and indirect fitness (Szulkin et al. 2013).

Optimally balancing mate choice between kin and non-kin requires kin recognition abilities (Waldman 1988; Sherman et al. 1997; Mateo 2004; Hepper 2005). Kin recognition is a prime mechanism allowing to avoid or prefer inbreeding across animals; other reported mechanisms include dispersal, polyandry, delayed maturation, and/or reproductive suppression (Pusey and Wolf 1996). For example, many group-living species use social familiarity based on prior association as proxy of close kin and prefer unfamiliar mates to avoid close inbreeding and loss of direct fitness (for review Pusey and Wolf 1996; Cheetham et al. 2008; for naked mole rats Clarke and Faulkes 1999). In contrast, inbreeding preference, likely to increase indirect fitness gains, has been observed in solitarily living harvest mice (Brandt and Macdonald 2011), group-living lizards (Bordogna et al. 2016), cichlids (Thünken et al. 2007), and fruit flies (Robinson, Kennington and Simmons 2012).). While behavioral avoidance or preference of inbreeding is empirically well documented, evidence of optimally balancing in- and out-breeding by selective mate choice is rare. Bateson's (1982) seminal study on quail is a classic example of optimal mate choice balance, using a no-choice experimental design. However, behavioral studies on mate choice based on genetic relatedness often lack supporting evidence of the adaptive value of in- and out-breeding in the animal in question. A notable exception is Keane (1990), who showed, by using a fourfold choice experimental design, that mate choice of white-footed mice aligns with in- and out-breeding depression effects. Moreover, in varying choice scenarios, animals should flexibly adjust in- and out-breeding preference according to the choice options, but adaptive flexible female choice has been rarely assessed. Richard et al. (2009) observed conditional inbreeding in dependence of age and corpulence in a semi-natural population of common lizards.

Here we addressed the combined predictions of optimal outbreeding (Bateson 1983) and kin breeding (Kokko and Ots 2006, Puurtinen 2011) theories in the plant-inhabiting predatory mite *Phytoseiulus persimilis*. These predators suffer from both close inbreeding and distant outbreeding (Atalay and Schausberger 2018): sib-mated females and females mated to non-kin from another population have ~25% lower lifetime reproductive success (LRS) than females mated to distant kin, i.e. mates from the same population (Atalay and Schausberger 2018). *P. persimilis* reproduce sexually, with males actively searching for females but, after mate encounter, females largely controlling whether copulation takes place or not (Schausberger et al. 2016, 2017). The female mating system is characterized as monandry to low level of polyandry, with one or two mates per female per life (Schausberger et al. 2016, 2017). Due to having only one or two mates, optimal female choice is decisive for LRS. *P. persimilis* are specialized predators of herbivorous spider mites (Sabelis 1985, McMurtry and Croft 1997), live in groups and possess kin recognition abilities, which are mechanistically based on prior association (Schausberger and Croft 2001, Enigl and Schausberger 2004, Schausberger, 2004, 2005, 2007). Depending on the behavioral and ecological contexts, they may use direct familiarity, allowing recognizing individuals met before, and indirect familiarity (dubbed phenotype matching), allowing also recognizing unfamiliar individuals bearing the same cue as those met before (Blaustein and Porter 1996, Mateo 2004), to discriminate kin and non-kin. We hypothesized that *P. persimilis* females should flexibly adjust mate choice depending on the choice options and the associated trade-offs in direct and indirect fitness (Atalay and Schausberger 2018). They should optimally balance mate choice by avoiding sib-mating when the alternative is distant kin but should prefer sib-mating when the alternative is non-kin.

2. Materials and methods

2.1 Experimental animals, population origins and rearing

Experimental individuals derived from two distinct laboratory-reared populations of *P. persimilis*. The laboratory populations were each founded by 50 to 100 specimens, field-collected in either Sicily or Greece, where *P. persimilis* is native (De Moraes et al. 2014). The populations were maintained in the laboratory for about 6 to 7 yrs before use in the experiments (Atalay and Schausberger 2018). Wide geographic separation suggests lacking or negligible genetic exchange between *P. persimilis* from Sicily and Greece. In the laboratory, the predators were reared in heaps of spider mite-infested bean leaves on artificial arenas consisting of plastic tiles (15 x 15 x 0.2 cm) resting on water-saturated foam cuboids in plastic boxes (20 x 20 x 6 cm) half-filled with water. Spider-mite infested bean leaves were added onto arenas in 2- to 3-day intervals (McMurtry and Scriven, 1965; Schausberger et al., 2016).

Two-spotted spider mites, *Tetranychus urticae,* were reared on whole bean plants *P. vulgaris,* grown at 23 ± 2 °C and 16:8 h L:D photoperiod. For both the pre-experimental procedure and experiments, the spider mites were either manually brushed from infested leaves onto fresh detached leaves, using a soft-haired paint brush, or using a mite brushing machine (BioQuip®, CA, USA) onto glass plates and then singly picked up and placed into the experimental acrylic cages using a fine red marten's hair brush.

All rearing and experimental units were kept in a climate chamber at 25 ± 1 °C, 65 ± 5 % RH and 16:8 h L:D photoperiod.

2.2 Pre-experimental procedures

To obtain and rear experimental animals we used detached bean leaf arenas infested by spider mites and closed acrylic cages. Each leaf arena consisted of a bean leaf placed upside down on a water-saturated foam cuboid (8 x 8 x 4 cm) covered with moist filter paper. The foam cuboid was kept in a small plastic box (10 x 10 x 6 cm) half-filled with water; the edges of the leaves were covered by strips of moist tissue paper to prevent the mites from escaping. Acrylic cages consisted of cylindrical cells of 15 mm diameter and 3 mm height, laser-cut into rectangular acrylic plates, closed with fine gauze at the bottom and a microscope slide on the upper side (Schausberger 1997).

To obtain even-aged eggs of *P. persimilis,* giving rise to females whose offspring were used in the experiments, gravid females were randomly withdrawn from the population rearing units and placed on detached leaf arenas infested by *T. urticae*. Eggs were daily collected with a fine brush, transferred to new leaf arenas infested by *T. urticae*, and reared there until the deutonymphal stage. Female deutonymphs were singly isolated inside acrylic cages that had been previously loaded with mixed spider mite stages, serving as prey for the predators, and cages were stored upside down on a grid inside plastic boxes, the bottom of which was covered by water to maintain elevated humidity inside the cages. After reaching adulthood inside the cages, each female was paired with a male randomly taken from its respective population. Offspring of these females were used as experimental individuals. Newly laid eggs were collected once per day and stored inside separate acrylic cages in the fridge at 8 °C. When approximately 20 to 25 eggs, ≤24 h old, were available per female, all her eggs were taken out from the fridge, split into two groups, each consisting of 10 to 15 eggs, and each group placed onto a separate detached leaf arena infested by spider mites. Siblings were reared to the deutonymphal stage within their group allowing to familiarize with siblings of this group but remaining unfamiliar with siblings of the other group. Splitting the sibling eggs into two separate groups thus allowed generating familiar and unfamiliar siblings. Deutonymphs were picked up from the leaves and singly isolated inside acrylic cages, which had been previously loaded with mixed spider mite stages, until reaching adulthood. After reaching adulthood, the individuals were sexed and ready for use in the experiments. Before use in experiments, adult males were colored with red or blue water color dots (Jolly; Brevillier Urban & Sachs, Vienna, AT) on their dorsal shields to make them distinguishable from the alternative mate option in female choice tests.

2.3 Experimental procedures

We conducted three choice experiments to unveil female mating preference in dependence of familiarity and genetic relatedness of the male mate options. To assess female choice, single virgin females from both populations, Sicily and Greece, were presented two virgin males differing in familiarity and/or degree of genetic relatedness to the female. Male mate options had one of three degrees of genetic relatedness to the female, close (siblings), distant kin (same population), or non-kin (different populations). Due to long-term rearing history in the laboratory (closed populations without immigration and emigration) we assumed coefficients of relatedness (r) of $r=0.5$ for siblings, $0<r<0.5$ for distant kin, and $r=0$ for non-kin. Siblings were either familiar or unfamiliar to the female, non-siblings were always unfamiliar. To start a choice experiment, the two males were placed inside an acrylic cage containing spider mites as prey and one virgin female was added. Cages were monitored in 20 min intervals, using a stero-microscope, until the first successful mate choice of the female occurred.Female choice was scored successful when one of the two males was clinging to the female from beneath in the venter to venter position for copulation (Amano and Chant, 1978). The first experiment aimed at finding out whether *P. persimilis* females are able to use direct familiarity (prior association) to recognize siblings and avoid mating with them when given a choice between a familiar and an unfamiliar sibling. The second experiment aimed at determining whether *P. persimilis* females are able to discriminate siblings from distant kin (same population). Siblings were either familiar or unfamiliar to the female. The third experiment aimed at determining whether *P. persimilis* females are able to discriminate siblings from non-kin (other population). Siblings were either familiar or unfamiliar to the female. The number of replicates was 15 (Greece) and 17 (Sicily) in experiment 1, 19 (Greece) and 16 (Sicily) for familiar sibling vs. unfamiliar distant kin and 16 (Greece) and 16 (Sicily) for unfamiliar sibling vs. unfamiliar distant kin in experiment

2, and 15 (Greece) and 16 (Sicily) for familiar sibling vs. unfamiliar non-kin and 16 (Greece) and 15 (Sicily) for unfamiliar sibling vs. unfamiliar non-kin in experiment 3. In each experiment each female, male and cage were used only once. Every female used in the experiments mated with one or the other male, no female was discarded from analysis.

2.4 Statistical analyses

Statistical analysis was carried out using IBM SPSS 23 (IBM, Armonk, NY, USA). Separate generalized linear models (GLM, binary logistic distribution) were run for each experiment and each choice scenario within experiments; all tests were two-sided. In the first step, to verify unbiasedness of the experimental setup, we compared color choice (red or blue) in dependence of the population origin of the females. In the second step, we compared familiarity choice (familiar vs. unfamiliar) or relatedness choice (sibling vs. distant kin or sibling vs. non-kin) in dependence of the population origin of the females. In the third step, we compared familiarity choice (familiar vs. unfamiliar) or relatedness choice (sibling vs. distant kin or sibling vs. non-kin) within each population.

3. Results

In no experiment and in no choice scenario did females of either population, Sicily and Greece, show a biased preference for red or blue males (GLM; intercept □$_1^2$ < 1.116, P > 0.29; population origin of female □$_1^2$ < 0.425, P > 0.51 for any scenario). In the first experiment, choice between familiar and unfamiliar siblings differed between females from Sicily and Greece (□$_1^2$ = 4.606, P = 0.03). While Sicily females did not discriminate familiar and unfamiliar siblings (□$_1^2$ = 0.524, P = 0.46), Greece females mated preferentially with unfamiliar siblings (□$_1^2$ = 4.612, P = 0.03) (Figure 1). In the second experiment, choice between familiar or unfamiliar siblings vs. unfamiliar distant kin did not differ between females from Sicily and Greece (familiar siblings: □$_1^2$ = 0.454, P = 0.50; unfamiliar siblings: □$_1^2$ = 0.000, P = 1.00). Females from both populations preferentially mated with unfamiliar distant kin (familiar siblings Sicily: □$_1^2$ = 3.621, P = 0.05, Greece: □$_1^2$ = 7.079, P = 0.008; unfamiliar siblings Sicily: □$_1^2$ =

6.626, P = 0.01, Greece χ_1^2 = 6.626, P = 0.01; Figure 2). In the third experiment, choice between familiar or unfamiliar siblings vs. unfamiliar non-kin differed between females from Sicily and Greece when the siblings were familiar (χ_1^2 = 8.174, P = 0.004) but did not differ when the siblings were unfamiliar (χ_1^2 = 0.928, P = 0.33). While Greece females did not discriminate familiar siblings from unfamiliar non-kin (χ_1^2 = 3.002, P = 0.08), Sicily females mated preferentially with familiar siblings (χ^2 = 5.241, P = 0.02). Females from both populations preferentially mated with unfamiliar siblings when the alternative option was unfamiliar non-kin (Sicily: χ_1^2 = 6.500, P = 0.01; Greece: χ_1^2 = 5.241, P = 0.02; Figure 3).

4. Discussion

Our study documents that predatory mite females *P. persimilis* optimally balance mate choice in dependence of the choice options and associated inclusive fitness trade-offs (Hamilton 1964ab¸ Atalay and Schausberger 2018). Such behavior meets the combined predictions of optimal kin- and out-breeding theories (Bateson 1983, Kokko and Ots 2006, Puurtinen 2011). Overall, females preferred distant kin, having an intermediate level of relatedness, to close kin (experiment 2), but preferred close kin to non-kin (experiment 3). Flexible female choice in *P. persimilis* is adaptive because distant kin provide higher inclusive fitness benefits than close kin (in relative comparison, direct fitness gains outweighing indirect fitness loss) whereas close kin provide higher inclusive fitness benefits than non-kin (in relative comparison, additional indirect fitness gain at similar direct fitness trade-offs) (Atalay and Schausberger 2018). Lacking preference of Greece females presented a binary choice of familiar sibling mates and unfamiliar non-kin mates is likely due to lower average relatedness and associated lower inbreeding level and lower achievable indirect fitness gains, in the Greece population than the Sicily population.

Experiments 1 and 2 revealed that females from Greece and Sicily differ in use of kin recognition mechanisms, with Greece females possessing finer-tuned kin recognition abilities than Sicily females. Greece females were able to use both direct familiarity (evident from experiment 1) and indirect familiarity (evident from experiments 2 and 3), whereas Sicily females only used indirect familiarity (dubbed phenotype matching; evident from experiments 1 to 3) to assess the mate options. Sicily females apparently familiarized with siblings during development, formed a generalized template of siblings and then discriminated siblings from distant kin and non-kin by indirect familiarity (Porter 1988; Blaustein and Porter 1996).However, use of a generalized template of siblings did not allow them to discriminate familiar and unfamiliar siblings (experiment 1). In contrast, Greece females could discriminate familiar and unfamiliar siblings. Accordingly, direct familiarity was a stronger indicator of close kin than indirect familiarity for Greece but not Sicily females. The observed interpopulation difference may be due to evolution of more advanced recognition abilities in the Greece than Sicily population and/or to a higher level of inbreeding in the Sicily than Greece population. Both populations had been closed and inbred in the laboratory for several years before the experiment, with bottleneck events likely differing between the two populations. Higher average level of genetic relatedness in the Sicily population may have decreased label variability, resulting in lower acceptance thresholds of Sicily females (Reeve 1989; Schausberger 2014).

In conclusion, many studies documented that kin recognition-based mate choice allows avoidance or preference of inbreeding (Pusey & Wolf 1996),both in vertebrates and invertebrates such as in common voles (Bolhuis et al. 1988), field crickets (Simmons 1991), ants (Keller and Passera 1993), termites (Shellman-Reeve 2001), cichlids (Thünken et al. 2007), or lizards (Bordogna et al. 2016). In contrast, few studies tested the hypothesis of balanced in- and out-breeding by kin recognition, linked to documented in- and out-breeding depression effects in the study animals (Keane 1990). Our study shows that animals can flexibly base female choice on inclusive fitness trade-offs of in- and out-breeding (Atalay and Schausberger 2018) in dependence of the mate options.

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Figure captions

Figure 1. Female choice between familiar and unfamiliar siblings. Mate preference of virgin predatory mite females *Phytoseiulus persimilis* from Sicily and Greece presented a binary choice of a familiar brother and an unfamiliar brother. Dotted vertical lines indicate the expected proportions at no preference. P-levels inside bars refer to GLM within each population and choice situation, assuming random choice; the P-level between bars refers to GLM between the two populations, assuming same choice. N = 15 (Greece) and 17 (Sicily).

Figure 2. Female choice between siblings and distant kin. Mate preference of virgin predatory mite females *Phytoseiulus persimilis* from Sicily and Greece presented a binary choice of a familiar or an unfamiliar brother and a distant kin male (coming from the same population). The dotted vertical line indicates the expected proportion at no preference. P-levels inside bars refer to GLM within each population and choice situation, assuming random choice; the P-level between bars refers to GLM between the two populations, assuming same choice. N = 19 (Greece) and 16 (Sicily) for familiar sibling vs. unfamiliar distant kin and 16 (Greece) and 16 (Sicily) for unfamiliar sibling vs. unfamiliar distant kin.

Figure 3. Female choice between siblings and non-kin. Mate preference of virgin predatory mite females *Phytoseiulus persimilis* from Sicily and Greece presented a binary choice of a familiar or an unfamiliar brother and a non-kin male (coming from another population). Dotted vertical line indicate the expected proportions at no preference. P-levels inside bars refer to GLM within each population and choice situation, assuming random choice; the P-level between bars refers to GLM between the two populations, assuming same choice. N = 15 (Greece) and 16 (Sicily) for familiar sibling vs. unfamiliar non-kin and 16 (Greece) and 15 (Sicily) for unfamiliar sibling vs. unfamiliar non-kin.

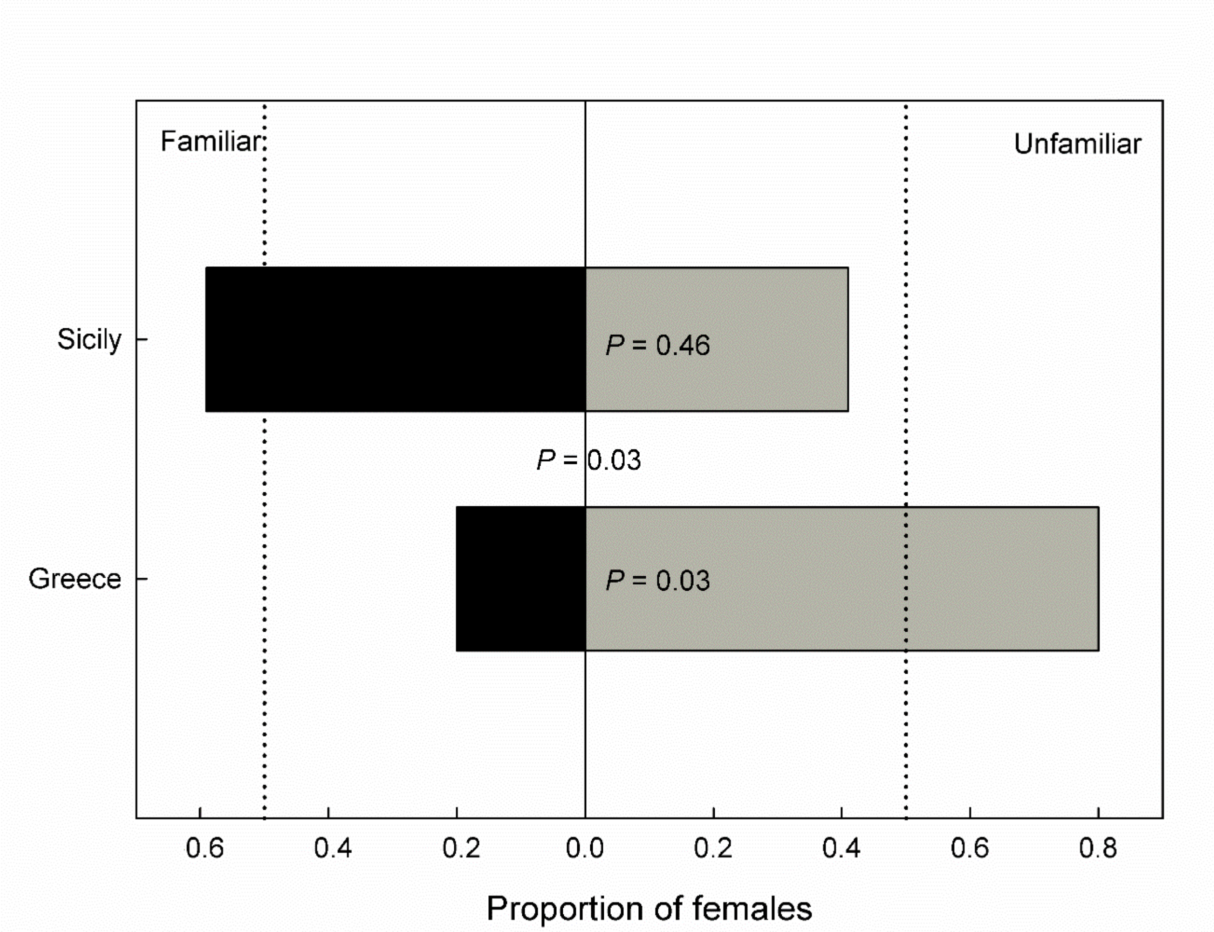
Familiar
Unfamiliar
Sicily
Greece
P = 0.46
P = 0.03
P = 0.03
0.6
0.4
0.2
0.0
0.2
0.4
0.6
0.8
Proportion of females

Figure 1

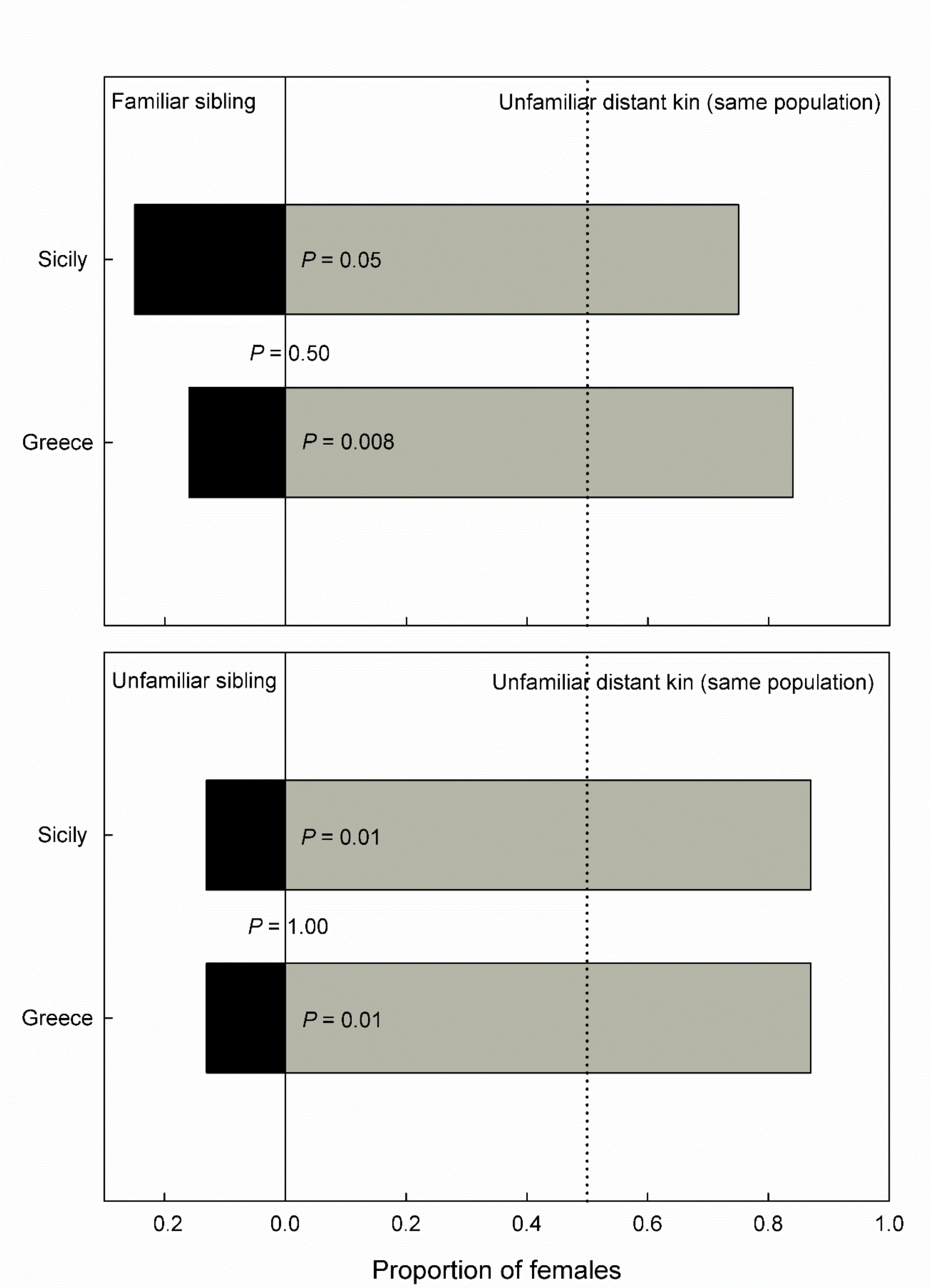
Familiar sibling
Unfamiliar distant kin (same population)
Sicily
P = 0.05
P = 0.50
Greece
P = 0.008
Unfamiliar sibling
Unfamiliar distant kin (same population)
Sicily
P = 0.01
P = 1.00
Greece
P = 0.01
0.2
0.0
0.2
0.4
0.6
0.8
1.0
Proportion of females

Figure 2

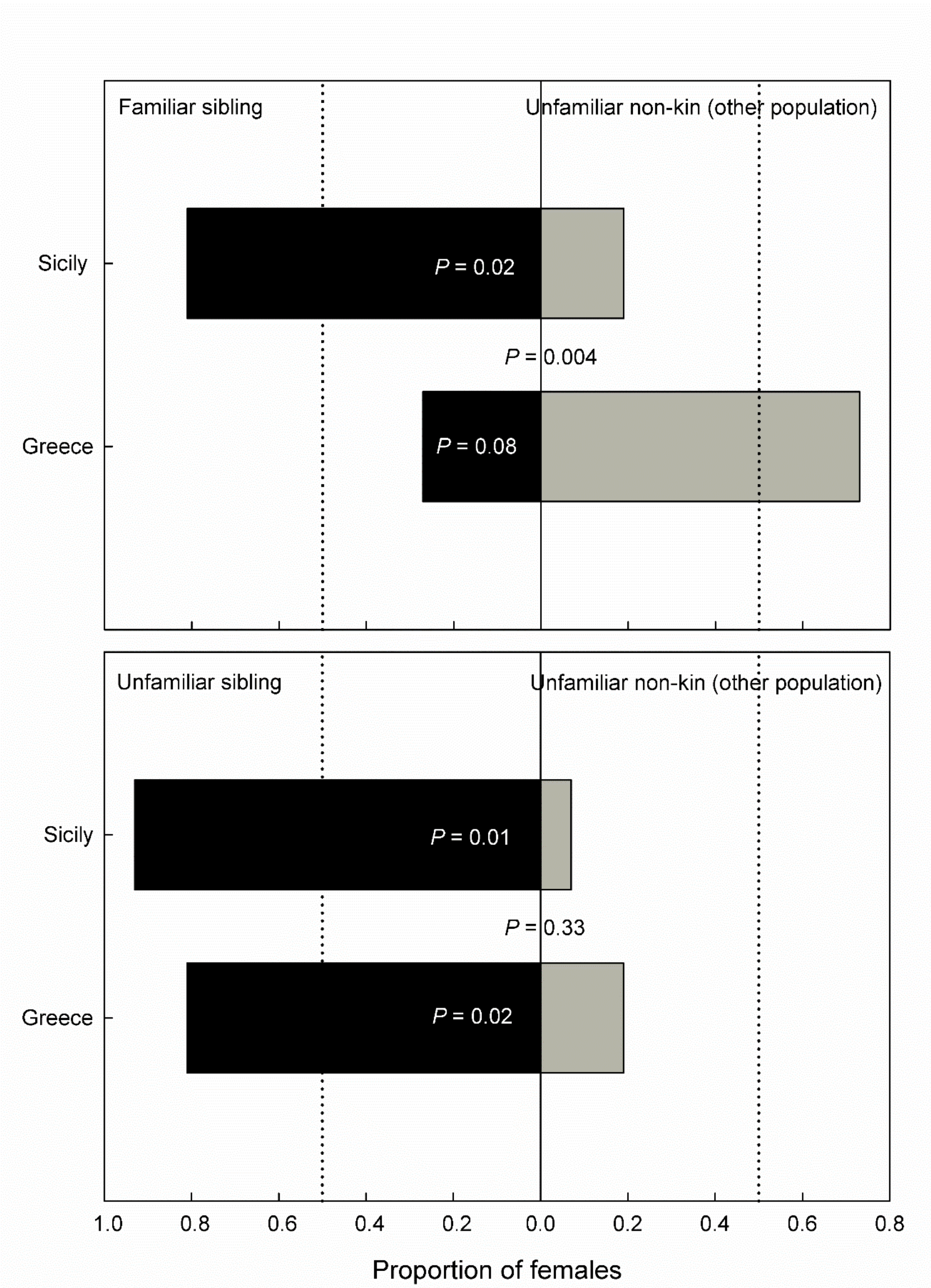
Familiar sibling
Unfamiliar non-kin (other population)
Sicily
Greece
P = 0.02
P = 0.004
P = 0.08
Unfamiliar sibling
Unfamiliar non-kin (other population)
Sicily
Greece
P = 0.01
P = 0.33
P = 0.02
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.4
0.6
0.8
Proportion of females

Figure 3

3 Presentations at scientific meetings related to this thesis

Çekin, D., Schausberger, P. (2017): Population level consequences of inbreeding in the predatory mite *Phytoseiulus persimilis*. Konferencja Naukowa „Rolnictwo XXI wieku – problemy i wyzwania". 28-29 March 2017, Kryzowa, Poland, p. 9.

Çekin, D., Schausberger, P. (2017): Hierarchical analysis of in- and out-breeding in haplodiploid predatory mites *Phytoseiulus persimilis*. XV international Congress of Acarology, 2-8 September 2018, Antalya, Turkey.

4 Abstract

The thesis consists of three inter-related but independent studies on in- and out-breeding effects in plant-inhabiting predatory mites *Phytoseiulus persimilis*. *Phytoseiulus persimilis* are haplo-diploid and pseudo-arrhenotokous; both females and males arise from fertilized eggs but males lose the paternal chromosome set during embryogenesis. In the first study, I assessed the fitness benefit-cost trade-offs between in- and out-breeding at the individual level. In the second study, I assessed the population dynamics of lineages from two origins of *P. persimilis*, Sicily and Greece, which, after founding, switched to obligatory inbreeding, at graded levels of genetic relatedness between founder mates (siblings, same population or different populations). In the third study, I assessed whether *P. persimilis* females can use a common inbreeding avoidance mechanism, that is, kin recognition; in detail, I evaluated the mate preference of females given binary choices according to familiarity and graded levels of genetic relatedness (close kin to distant kin to non-kin) of the mate options.

The first study supports the optimal outbreeding theory and existence of an optimal balance between in- and out-breeding. Mating at intermediate levels of genetic relatedness (same population but no siblings) provided for the highest fitness gains. I assessed the mating behavior of *P. persimilis* at different mate relatedness levels, and examined the number and sex ratio of their offspring. In the experiment, I used females from iso-female lines of two populations (Sicily and Greece), which mated with either a sibling, a male from the same population or a male from the other population. This study revealed that females mating with a male from the same population produced more eggs over longer time than females mating with a sibling or with a male from the other population. Mating duration was longer, and the proportion of daughters was higher, in sibling couples than couples of the same population and couples from different populations. Mating latency was unaffected by the genetic relatedness of mates.

The second study shows the consequences of founder in- and out-breeding levels for the population dynamics of chronically inbreeding lineages over four generations. Founder females were mated with either a sibling male, a male from the same population (but from a different family) or a male from the other population. After founding, experimental lineages were subjected to chronic inbreeding and increasing food stress. In the short run (F_1 generation), females mated to a male of intermediate level of genetic relatedness (same population) performed the best; such females produced more offspring than females mated to a sibling or to a male from the other population. In the medium to long term (F_2 generation onwards), females mated to an unrelated male performed better than females mated to a sibling or to a male from the same population. This study suggests that distantly outbred founder females introduced higher allelic variability and lower homozygosity levels, counterbalancing inbreeding depression from the F_2 generation onwards.

In the third study, I assessed the mate preference of females given binary choices according to familiarity and genetic relatedness of the available male options, based on the predictions of optimal outbreeding and (k)in breeding theories. (K)in breeding theory suggests that, at similar direct fitness costs, females should prefer to mate with close kin over non-kin because of inclusive fitness gains. Optimal outbreeding theory suggests that females should prefer mates at intermediate levels of relatedness for an optimal balance between in- and out-breeding. Virgin females were presented binary choices between brothers and distant kin or non kin. Females from the Greece population preferred to mate with their unfamiliar brothers. Females of both origins preferred to mate with males from same population over their brothers. However, they preferred to mate with brothers over non-kin. This study revealed that females can flexibily base their mate preferences on inclusive fitness trade-offs of in- and out-breeding in dependence of the mate options.

Overall, in- and outbreeding depression have been rarely studied in haplodiploid organisms. My thesis aimed at filling this gap in knowledge and providing a basis for future works. My thesis may contribute to improve the use of *P. persimilis* and other pseudo-arrhenotokous predatory mite species in augmentative and classical biological control of herbivorous mites in agricultural settings.

5 Zusammenfassung

Diese Dissertation umfasst drei unabhängige aber miteinander verbundene Studien über die Auswirkungen der In- und Auszucht bei pflanzenbewohnenden Raubmilben *Phytoseiulus persimilis*. *Phytoseiulus persimilis* sind haplo-diploid und pseudo-arrhenotokous; beide Geschlechter, Weibchen und Männchen, entstehen aus befruchteten Eiern, aber die Männchen verlieren den väterlichen Chromosomensatz während der Embryogenese. In der ersten Studie untersuchte ich die Fitnesseffekte der In- und Auszucht auf individueller Ebene. In der zweiten Studie habe ich das Populationswachstum und die Dynamik von Linien aus zwei Herkünften von *P. persimilis*, Sizilien und Griechenland, ausgewertet. Die Linien wurden von Weibchen mit unterschiedlicher genetischer Verwandtschaft ihrer Paarungspartner gegründet (Paarung zwischen Geschwistern, Individuen der gleichen Population oder Individuen verschiedener Populationen) und waren nach der Gründung obligatorischer chronischer Inzucht ausgesetzt. In der dritten Studie wurde ausgewertet, ob *P. persimilis* Weibchen Verwandtenerkennung zur Vermeidung von Inzucht verwenden können; im Detail wurden die Partnerpräferenzen von Weibchen ausgewertet, die in binären Wahlsituationen zwei mögliche Paarungspartner mit unterschiedlicher Vertrautheit und abgestuften Graden der genetischen Verwandtschaft (eng verwandt, entfernt verwandt, nicht verwandt) angeboten bekamen.

Die erste Studie unterstützt die Theorie der optimalen Auszucht (*optimal outbreeding*) und die Existenz einer optimalen Balance zwischen In- und Auszucht. Paarung zwischen individuen mit intermediärem Grad der genetischen Verwandtschaft (gleiche Population aber keine Geschwister) sorgte für die höchsten Fitnessgewinne. Ich wertete das Paarungsverhalten von *P. persimilis* bei verschiedenen Partnern und Verwandtschaftsgraden aus, und untersuchte die Anzahl und das Geschlechterverhältnis ihrer Nachkommen. Im Experiment wurden Weibchen aus zwei Populationen (Sizilien und Griechenland) verwendet, die sich entweder mit einem Bruder, einem Männchen aus der gleichen Population oder einem Männchen aus der anderen Population verpaarten. Meine Studie ergab, dass Weibchen, die sich mit einem

Männchen aus der gleichen Population verpaarten, mehr Eier über längere Zeit produzierten als Weibchen, die sich mit einem Bruder oder einem Männchen aus der anderen Population verpaarten. Die Kopulationsdauer war länger und der proportionale Töchteranteil war bei Geschwisterpaaren höher als bei Paarungspartnern aus der gleichen Population und Paarungspartnern aus unterschiedlichen Populationen. Die Kopulationslatenz war von der genetischen Verwandtschaft der Paarungspartner nicht betroffen.

Die zweite Studie zeigt die Konsequenzen des In- und Auszuchtgrades von Gründerweibchen auf die Populationsdynamik von geschlossen, und somit chronische Inzucht betreibenden, Linien über vier Generationen. Die Gründerweibchen wurden entweder mit einem Bruder, einem Männchen aus der gleichen Population (aber kein Bruder) oder einem Männchen aus der anderen Population verpaart. Kurzfristig (F_1 Generation) schnitten die Weibchen die sich mit einem Männchen von intermediärer genetischer Verwandtschaft (gleiche Population aber kein Bruder) verpaart hatten, am besten ab; solche Weibchen produzierten mehr Nachkommen als die Weibchen die sich mit einem Bruder oder einem Männchen aus der anderen Population verpaart hatten. Mittel- bis lang-fristig (ab der F_2 Generation) zeigten Weibchen, die sich mit einem genetisch nicht verwandten Männchen verpaart hatten, eine bessere Leistung bezüglich Nachkommenzahl, als Weibchen die sich mit einem Bruder oder einem Männchen aus derselben Population verpaart hatten. Diese Studie legt nahe, dass Kreuzung zwischen nicht verwandten Paarungspartnern eine höhere Variabilität an Allelen und einen geringeren Grad der Homozygotie in den daraus hervorgehenden Linien bewirkt. Diese Phänomene milderten die Inzuchtdepression ab der F_2 Generation.

In der dritten Studie habe ich die Paarungspartnerpräferenz der Weibchen in binären Wahlsituationen, unter kombinierter Berücksichtigung der Vorhersagen der Theorien der optimalen Auszucht und der Verwandtenselektion, ausgewertet. Die Theorie der Verwandtenselektion prognostiziert, dass Weibchen, bei ähnlich hohen direkten Fitnesskosten, nahe verwandte Paarungspartner gegenüber nicht-verwandten

Paarungspartnern bevorzugen sollen, da dies zu einem Gewinn der inklusiven Fitness führt. Die Theorie der optimalen Auszucht prognostiziert, dass Weibchen Paarungspartner mit mittleren Verwandtschaftsgraden bevorzugen sollen, um eine optimale Balance zwischen In- und Auszucht zu erreichen. Jungfräuliche Weibchen konnten in binären Wahlsituationen zwischen vertrauten oder nicht vertrauten Brüdern und entfernt verwandten Männchen oder nicht-verwandten Männchen wählen. Weibchen aus der Griechenland Population verpaarten sich bevorzugt mit nicht vertrauten Brüdern. Die Weibchen beider Herkünfte bevorzugten Männchen aus der eigenen Population gegenüber Brüdern als Paarungspartner. Allerdings bevorzugten sie Brüder gegenüber nicht verwandten Männchen. Diese Studie ergab, dass Weibchen flexible Partnerpräferenzen, in Abhängigkeit der Partneroptionen und je nach den zu erwartenden Kosten/Nutzen in inklusiver Fitness, haben.

Insgesamt wurden In- und Auszuchtdepressionen bei haplodiploiden Organismen selten untersucht. Meine Dissertation zielte darauf ab, diese Wissenslücke zu füllen und eine Basis für zukünftige weiterführende Studien zu bieten. Meine Dissertation sollte einen Beitrag zur Verbesserung der Verwendung von *P. persimilis* und anderen pseudo-arrhenotoken Raubmilbenarten in der Biologischen Kontrolle von herbivoren Milben leisten.

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7 Curriculum Vitae

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Name: Demet Çekin

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Professional Experience

03.2012 - 09.2013	Project assistant at University of Süleyman Demirel, Isparta-Turkey
12.2011 - 03.2012	Consultant agricultural engineer at Akgül Tarim, Antalya-Turkey

University Education

01.2014 - present	Doctorate of Natural Resources and Life Sciences at the University of Natural Resources and Life Science, Vienna, Department of Crop Sciences Topic: In- and outbreeding effects in the predatory mite *Phytoseiulus persimilis*
02.2012-09.2013	Master studies in Department of Plant Protection with specialization Entomology at University of Süleyman Demirel, Isparta-Turkey Thesis: The life table of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on different tomato varieties

09.2007-09.2011	Bachelor studies in Agricultural Engineering in Faculty of Agriculture with specialization Plant Protection at Gaziosmanpasa University, Tokat-Turkey Thesis: Interactions between Rape Pollen Beetle *(Meligethes aeneus*) and volatile organic compounds on Rape
09.2010-08.2011	Semester Abroad (Erasmus Student exchange program) in Agronomy at University of Technology and Life Sciences, Bydgoszcz-Poland

Fellowship/Internships

06.2010-08.2010	Internship at University of Gaziosmanpasa, Tokat
08.2010-09.2010	Internship at Institute of Biological Control Research, Adana

Vocational Training

10.2011-11.2011	Sales consultancy of pesticides and fertilizers at Güvercin Gübre A.S, Antalya

School Education

09.2004-06.2007	High School: Natural Sciences and Math at Karapınar High School, Karapınar - Konya
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