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Inbreeding affects personality and fitness of a leaf beetle

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Habitat loss and fragmentation, which are mostly anthropogenically caused, can lead to decreasing population sizes for many species. Consequently, the risk of inbreeding increases and inbreeding depression can be induced, which is likely to lead to a decline in fitness in inbred individuals. We investigated effects of inbreeding on fitness-related traits during ontogeny of a wild leaf beetle, *Phaedon cochleariae*, population. We also examined whether the behavioural phenotype, that is, personality, of adult beetles is affected by inbreeding, sex and/or age. We found evidence for inbreeding depression both at the larval stage and in adulthood. We tested the behaviour twice during adulthood and found that beetles show personality, that is, activity, exploration and boldness traits were repeatable and consistent across contexts. Moreover, the personality of inbred and outbred beetles differed significantly. In detail, boldness and exploration traits were affected by inbreeding, partly interacting with the beetles' sex or age. Most notably, inbred beetles were bolder in an unprotected environment than outbred beetles. Inbred individuals may be more risk prone in specific situations, because they have less to lose, since they reproduce less. As well as inbreeding effects on fitness-related traits, the results also show that the personality of arthropods can be affected by inbreeding. This is potentially related to inbreeding depression and thus to fitness declines in inbred individuals. Overall, decreased fitness combined with personality alterations of inbred individuals might affect the further development and survival of populations and thus possibly also (interactions with) other trophic levels of the ecosystem.

Human activities, especially agriculture, transform and fragment habitats (Chaplin-Kramer et al., 2015; Schmitz et al., 2014). Recent estimates suggest there will be a large increase in land under agriculture in the next few decades (Kareiva & Marvier, 2012; Schmitz et al., 2014). The consequence of this development is an ongoing loss and/or deterioration of natural habitats, which can entail a decrease in, and fragmentation of, populations in many species living in these environments (Agnarsson, Aviles, & Maddison, 2013; Bates et al., 2014). As a result, the risk of inbreeding increases (Agnarsson et al., 2013; Bates et al., 2014), which can lead to inbreeding depression resulting from increased homozygosity (Keller & Waller, 2002). Thereby, recessive deleterious alleles can be unmasked and advantageous heterozygosity is lost (Charlesworth & Willis, 2009). As a consequence, inbreeding depression causes a decline in fitness (Saccheri, Brakefield, & Nichols, 1996) in many (arthropod) species (Edvardsson, Rodriguez-Munoz, & Tregenza, 2008; Lihoreau, Zimmer, & Rivault, 2007; Liu, Tu, He, Chen, & Xue, 2014; Tien, Massourakis, Sabelis, & Egas, 2011). Furthermore, the ability of a population to adapt to changing environments might be restricted (Franke & Fischer, 2013). To avoid these inbreeding costs, many species have pre- or postcopulatory inbreeding avoidance mechanisms (Bretman, Newcombe, & Tregenza, 2009; Liu et al., 2014; Thomas & Simmons, 2011; Wedell, Gage, & Parker, 2002) as well as specific dispersal strategies (Pusey & Wolf, 1996).

While inbreeding effects on fitness-related traits of many arthropod species have been thoroughly investigated (Radwan, 2003; Tan, Løvlie, Pizzari, & Wigby, 2012), the impacts of inbreeding on the behavioural phenotype and especially on the personality of inbred individuals remain unexplored, not only in arthropods, but also in vertebrates. This is especially surprising as behavioural changes of animals caused by inbreeding might be the first visible responses to environmental changes, such as habitat loss or fragmentation. So far, only a few studies have tested for inbreeding effects on single behavioural traits. For example, the mate choice or preference behaviour of insects can be affected by the breeding status of either females or males (Michalczyk et al., 2011; Pilakouta & Smiseth, 2017; Pölkki, Krams, Kangassalo, & Rantala, 2012). Moreover, the dispersal of two-spotted spider mites, *Tetranychus urticae*, is influenced by the interaction between inbreeding and the degree of relatedness of individuals living in a

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given group (Bitume et al., 2013). Inbred burying beetle, *Nicrophorus vespilloides*, males are more risk prone during competition for limited resources than outbred individuals (Richardson & Smiseth, 2017). However, inbreeding can be expected to affect not only single behavioural traits but also the personality of an organism, that is, behavioural differences between individuals of a population that are consistent over time and across different contexts (Dall, Houston, & McNamara, 2004; Stamps & Groothuis, 2010). The expression of a certain personality can be related to the reproductive value of an individual (Clark, 1994; Tremmel & Müller, 2013). Thus, possible inbreeding effects on personality might be related to inbreeding depression and thus to decreased fitness, for example characterized by a reduced reproductive output of inbred compared to outbred individuals.

In this study, we investigated the effects of inbreeding on fitness-related traits during ontogeny and on the adult behavioural phenotype, that is, personality, of mustard leaf beetles, *Phaedon cochleariae*, from the third generation of a wild-caught population (hereafter termed 'wild' population). The behavioural phenotype, that is, the expression of distinct activity-, exploration- and boldness-related behavioural traits (Müller, Küll, & Müller, 2016; Tremmel & Müller, 2013), of inbred and outbred beetles was determined twice between days 5 and 14 of the adult's life.

Selective matings in *P. cochleariae*, which were reared for more than 25 generations in the laboratory, revealed that the hatching rate is significantly lower for inbred than outbred larvae (Müller & Müller, 2016). Consequently, we hypothesized that the inbred offspring of the wild population would also suffer from inbreeding depression, probably throughout their ontogeny. Furthermore, we expected that the behaviour of the adults used in this experiment would be repeatable and consistent across the tested behavioural contexts and thus that the beetles would show personality, as found in 10–45-day-old adult *P. cochleariae* of a laboratory colony (Müller & Müller, 2015; Tremmel & Müller, 2013). Moreover, we hypothesized that the personality of inbred and outbred individuals would differ, potentially due to the close link between personality and fitness (Smith & Blumstein, 2008), which is likely to be lower in inbred than in outbred individuals. In addition, we expected sex- and age-dependent impacts on the behavioural phenotype, which have been demonstrated before (Müller et al., 2016; Müller & Müller, 2015). In detail, we hypothesized that inbred beetles would be less active and explore less than outbred beetles, because inbreeding can decrease the metabolic scope, as has been found in a cricket species (Ketola & Kotiaho, 2009), which in turn may reduce locomotion. We also hypothesized that inbred beetles might be bolder than outbred beetles as they have a lower reproductive output and thus less to lose, which would be in line with the asset protection principle (Clark, 1994).

METHODS

Leaf Beetle Origin and Rearing

Approximately 120 adult *P. cochleariae* (Coleoptera: Chrysomelidae) beetles were captured along the Furlbach in Bielefeld-Senne, Germany, from three watercress, *Nasturtium officinale*, patches that were each 30–50 m apart in 2016. In the laboratory, these beetles were reared in three ventilated plastic boxes (20×20 cm and 6.5 cm high), separated by their capture location, in a climate cabinet under constant conditions (20 °C, 65% relative humidity, 16:8 h light:dark). The beetle generation caught in the field [parental (P) generation] was fed ad libitum with leaves of nonflowering 6–8-week-old watercress (seeds from Volmary GmbH, Münster, Germany) and cabbage plants, *Brassica rapa* ssp. *pekinensis* var. Michihili (seeds from Kiepenkerl, Bruno Nebelung GmbH, Konken, Germany) that were grown in pots (12 cm diameter) filled with composted soil in a greenhouse (60% relative humidity, 16:8 h light:dark). Eggs of the P generation were collected from cabbage leaves and, from the first laboratory generation (F1) on, beetles were exclusively reared on middle-aged cabbage leaves for practical reasons and to ensure a higher comparability to previous studies on this beetle. F1 larvae were reared in the same sort of plastic boxes, separated by the capture location of their parents, until pupation.

Experimental Set-up

To test the influence of inbreeding (inbreeding treatment: inbred versus outbred individuals) on larval development, reproductive output and adult behavioural phenotype of *P. cochleariae*, the following experiment was set up. Randomly chosen, freshly hatched virgin females and males of the F1 generation that descended from different rearing boxes and thus from different capture sites were used as initial breeding pairs. The adults of the F1 generation and the larvae and adults of the subsequent generations (F2 and F3) were reared in petri dishes lined with moistened filter paper. Beetles were fed ad libitum with discs (2.5 cm diameter) of middle-aged cabbage leaves, which were exchanged every to every other day. Eggs of each F1 pair were collected and larvae of the F2 generation were reared in sibling groups of about 15–20 larvae per large petri dish (9.5 cm diameter). After pupation, beetles were separately reared in small petri dishes (5.5 cm diameter). After sex determination, breeding pairs of one female and one male descending from either the same (sibling pairs) or different parents (nonsibling pairs) were set up in small petri dishes. These parents were considered as 'families'. Once females of the F2 generation started to lay more than 10 eggs per day, at days 10–12 of their adult life, their eggs were collected, separately for each female. Inbred and outbred larvae of the F3 generation, which descended from either sibling or nonsibling parents, were reared at a density of 8–10 individuals per large petri dish and were used as the experimental generation. Inbred and outbred adults of the F3 generation were reared in small petri dishes (one adult per dish) from the day of adult emergence to the last behavioural test (day 14). Afterwards, the inbred and outbred females of this generation were mated with an outbred male of a different family. The adult beetles of the F3 generation descended from nine different families.

In summary, all beetles of the three generations were reared alone until a mating partner of the opposite sex was introduced to their petri dish. Thus, the mating partner of every beetle was controlled to prevent inbreeding events prior to the experiment. Based on this breeding scheme for three generations, the parents of individuals considered as 'inbred' offspring were all full siblings and the parents of individuals considered as 'outbred' offspring were neither full nor half siblings nor cousins.

Development and Reproduction

To determine the hatching rate of inbred and outbred larvae of the F3 generation, we counted the eggs of the sibling and nonsibling parents of the F2 generation and subsequently the larvae (F3 generation) that hatched from these eggs (after 6–7 days). Larval development time was determined from the day of larval hatch to adult emergence of the F3. We also recorded the survival rate of inbred and outbred F3 larvae to adulthood per rearing petri dish.

To test for effects of the female inbreeding treatment on reproductive output, randomly chosen inbred and outbred females were mated with an outbred male of a different family (of the F3) after the last behavioural test was conducted (day 14). Owing to the limited number of males ($N = 30$), only half of the females were