GÖTEBORGS UNIVERSITET

INSTITUTIONEN FÖR BIOLOGI OCH MILJÖVETENSKAP

Evolutionary Genetics and Consequences of Inbreeding in Sand Lizards (*Lacerta agilis*)

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Akademisk avhandling för filosofie doktorsexamen i naturvetenskap, inriktning biologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 28 maj 2021 kl. 10:00 i Hörsalen, Botanhuset, Institutionen för biologi och miljövetenskap, Carl Skottsbergs gata 22, Göteborg.

Opponent är Professor Lukas Keller, Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland.

ISBN: 978-91-8009-340-8 (Print)

ISBN: 978-91-8009-341-5 (PDF)

Electronic version: http://hdl.handle.net/2077/68194

Printed by Stema Specialtryck AB

Cover picture by Erik Wapstra

To my mother

Abstract

Inbreeding is a well-known phenomenon in evolutionary and conservation biology. In the 19th century, Charles Darwin demonstrated the detrimental effects of inbreeding in plants, followed by over a century of extensive research including various animal and plant taxa. The effects of consanguineous matings are particularly pronounced in fitness-determining traits, such as life-history and sexually selected traits. Accordingly, a large body of literature has developed on traits that are directly associated with fitness, such as survival and reproductive output. Sexually selected traits have however received less attention. Due to its detrimental effects on fitness, inbreeding is often associated with the evolution of inbreeding avoidance mechanisms. This logical expectation is, however, not always met, with a number of studies reporting an absence of inbreeding avoidance in populations affected by inbreeding depression. The inconsistency in reports of inbreeding avoidance among studies is likely due to a balance between the costs and benefits of inbreeding, rendering predictions exceedingly difficult.

I used long-term data (collected over a decade) from a Swedish population of sand lizards (*Lacerta agilis*) to measure the effects of inbreeding on two key ontogenetic stages, embryonic development and first year post-hatching, survival, and a trait expressed in adult males, the intra-sexually selected green badge. The badge is developed laterally by males during the mating season, and varies in size and pigment saturation among individuals. This area of green nuptial coloration functions as a signal of fighting ability during male-male interactions, for access to females. I studied the mating pattern in the population, for signs of inbreeding avoidance. In addition, I evaluated the role of the major histocompatibility complex (MHC) as a cue of kinship. Inbreeding significantly affected hatching success, but not first year survival. Both structural and pigmentary components of the badge were affected by individual-level heterozygosity (size, pigment saturation, and brightness). Finally, no evidence of inbreeding avoidance was found, but parental pairs were more dissimilar at the MHC than expectations under random mating. Thus, parental pair similarity at the MHC does not appear to reliably reflect genome-wide similarity, in the Asketunnan population.

The effects of inbreeding in natural populations are difficult to measure. Traditionally, survival and reproductive success have been used to measure inbreeding depression. However, the results of these approaches can be confounded by various factors, such as environmental heterogeneity and parental effects. Moreover, the magnitude of inbreeding depression may vary according to the considered ontogenic stage. Thus, an alternative would be to measure inbreeding depression using cellular function, which should reflect the accumulation of stress experienced by an individual over long periods of time. Telomeres offer this possibility, as their attrition rate is linked to somatic stress. Thus, one would expect inbred individuals to have short telomers, relative to less inbred conspecifics. Surprisingly, no such effect was found in the study population. The only significant effect of inbreeding on telomere length that was found is a deviation from the mean maternal heterozygosity that is associated with shorter hatchling telomeres, suggesting stabilizing selection.

The final objective of the thesis was to estimate the additive genetic variance, and heritability, of badge size. The impetus for this work is the “lek paradox” theory, which predicts the erosion of additive genetic variance in fitness-determining traits, due to strong directional selection. In addition, the importance of genic capture for maintaining additive genetic variance in badge size was assessed by estimating additive genetic variance in body condition. The results of these analyses show a significant estimate of additive genetic variance in badge size, but not in body condition, which appears to be environmentally determined. This suggests that age and sex-dependent selection, combined with a complex genetic architecture that underlies multiple colour components, may explain the persistence of additive genetic variance in badge size in the sexually dichromatic sand lizard.

Populärvetenskaplig sammanfattning

Inavel är ett välkänt fenomen som påverkar många djur- och växtarter negativt. Inom drabbade populationer utsätts ofta inavlade individer för skadliga effekter, såsom missbildningar eller förkortad livslängd, vilket i sin tur leder till hot mot populationens uthållighet som helhet. På grund av dessa negativa effekter undviks ofta inavel, med hjälp av en rad olika signaler, såsom att undvika individer med liknande immunförsvarsgener (så kallade MHC-gener). Detta är dock inte alltid fallet. Vissa inavlade individer undviker inte parning med släktingar. Dessutom, i vissa fall, föredrar till och med organismer att para sig med släktingar (för att dom hjälper varandra att föra samma gener vidare till nästa generation). Jag studerade därför inavel i en population av sandödlor (*Lacerta* agilis) från Asketunnan, Sverige, bland annat i ett sammanhang av hur inavel styr parningsmönster och om man kan se tecken på undvikande eller preferens av mer eller mindre närbesläktade partners. Slutligen, undersökte jag hur inavel påverkar hanarnas gröna parningsfärg, samt uppskattade ärftligheten av just parningsfärgen (som ett indirekt mått på sexuell selektion, dvs vad som styr organismers reproduktionsframgång relativt sina konkurrenter). Hanarnas gröna färg på kroppssidorna under parningssäsongen används som information om rivalers kampförmåga. Vinnarna av sådana kamper får tillgång till honor och därmed ökar de sina chanser till parning och att föra sina gener vidare till nästa generation. Således, om inavel har negativa effekter på den gröna färgen, skulle detta leda till minskad parningsframgång och selektion skulle motverka inavel via kamper och – kanske - partnerval.

Mina resultat visade på negativa effekter av inavel på kläckningsframgång av ägg, men inte första årets överlevnad hos nyckläkta sandödlor i deras naturliga miljö. Dessutom fann jag endast små effekter av inavel på hanarnas gröna färg och dess ärftlighet. Däremot fann jag inga effekter på telomerlängd., de icke-kodande DNA-regionerna på kromosomernas ändar som ibland har använts för att mäta inavelseffekter. Man förväntar sig kanske att individer med störd cellfunktion (som är fallet hos inavlade individer) skulle ha svårt att upprätthålla kostsamt underhåll av telomerer, men så var inte fallet hos sandödlorna i vår population. Trots ovannämnda negative effekter undviks inte inavel i populationen. Däremot, parningen var skevt fördelad mot individer med andra immunförsvarsgener än ens egna, men däremot inte likhet på andra delar av arvsmassan.

Resultaten av min avhandling visar vikten av att överväga flera livshistoriska stadier under bevarandeinsatser i populationer som påverkas av inavel. Mer forskning behövs för att fastställa vikten av inavel som drivkraft för parningsmönster i naturliga populationer, samt fastställa vilka signaler som används för att särskilja släktingar från obesläktade individer. Jag uppmuntrar till ett försiktigt tillvägagångssätt när man använder begränsade genetiska segment (såsom MHC-gener) för att härleda likheter i hela DNA genomet i bevarandebiologi där man eftersträvar att undvika inavel- och utavelssdepression (dvs med negativa effekter av för avlägset besläktade individer).

Chapters

This thesis is based on the following chapters:

Chapter I: Bererhi, B., Wapstra, E., Schwartz, T. S. & Olsson, M. 2019. Inconsistent inbreeding effects during lizard ontogeny. *Conservation Genetics*, 20, 865-874.

Chapter II: Bererhi, B., Lindsay, W. R., Schwartz, T. S., Wapstra, E. & Olsson, M. Effects of inbreeding on a sexually-selected trait, the sand lizard badge. *Manuscript*.

Chapter III: Bererhi, B., Duchesne, P., Schwartz, T. S., Ujvari, B, Wapstra, E. & Olsson, M. Inbreeding, major histocompatibility complex, and disassortative mating in sand lizards. *Submitted*.

Chapter IV: Olsson, M., Bererhi, B., Miller, E., Rollings, N., Lindsay, W. R. & Wapstra, E. Inbreeding effects on telomeres in hatchling sand lizards (*Lacerta agilis*): just a family affair? *Manuscript*.

Chapter V: Lindsay, W. R., Bererhi, B., Ljungström, G., Schwartz, T. S., Wapstra, E. & Olsson, M. Maintenance of additive genetic variance in sexually selected badges in sand lizards (*Lacerta agilis*) cannot be explained by genic capture. *Manuscript*.

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Examiner: Professor Staffan Andersson, Department of Biological and Environmental Sciences, University of Gothenburg.

The following paper was published during the course of the thesis.

Lindsay, W. R., Andersson, S., Bererhi, B., Höglund, J., Johnsen, A., Kvarnemo, C., Leder, E. H., Lifjeld, J. T., Ninnes, C. E., Olsson, M., Parker, G. A., Pizzari, T., Qvarnström, A., Safran, R. J., Svensson, O. & Edwards, S. V. 2019. Endless forms of sexual selection. *PeerJ*, 7, e7988.

Contents

Background

The common denominator of most of this thesis is inbreeding in a population of sand lizards (*Lacerta agilis*), in Aketunnan, Sweden. I address a number of important questions within the field of evolutionary genetics, including the impact of inbreeding on fitness-determining traits (**chapter I, II and IV)** and its effects on the population's mating system, focusing on kin recognition and the nature of the cues that allow it (**chapter III)**. In the context of sexual selection, I estimate the additive genetic variance and heritability of a male colour display- a patch of bright green coloration, displayed during male-male interactions, that functions as a signal 'badge' of fighting ability (Olsson 1994). Armed with these parameter estimates, I address a well-known phenomenon associated with the "lek paradox" (Andersson 1994; Pomiankowski and Moller, 1995; Kirkpatrick and Ryan, 1999; Rowe and Houle, 1996), the erosion of additive genetic variance of sexually selected traits under strong directional selection. Surprisingly, additive genetic variance is often maintained in sexually selected traits (Houle 1992; Pomiankowski and Moller, 1995; Rowe and Houle 1996), possibly owing to the 'capture' during trait evolution of additional sources of variance in body condition (Rowe and Houle, 1996; Griffith et al., 1999). I test this prediction by estimating the additive genetic variance in body condition and its effect on badge size, using animal models (**chapter V**). The following sections summarize the theoretical and empirical knowledge upon which the thesis is based.

Inbreeding and inbreeding depression

Inbreeding is a well-known phenomenon in evolutionary and conservation biology (Darwin 1868; Charlesworth and Charlesworth, 1987). In the 19th century, Charles Darwin observed wide-spread mechanisms associated with the avoidance of self-fertilization in plants. He then established their importance by showing that self-fertilization produced progeny with inferior trait values, relative to cross-fertilized ones. These traits included height, flowering date, weight, and number of seeds (Darwin 1868, 1876). Since then, the detrimental effects of inbreeding have been documented in many taxa (Keller et al., 1994; Olsson et al., 1996a; Crnokrak and Roff, 1999; Slate et al., 2000; Amos et al., 2001; Keller and Waller, 2002; Huisman et al., 2016), highlighting the important influence of inbreeding on individual and population viability.

- Definition of inbreeding

Inbreeding can have severe consequences on fitness. However, its definition did not seem to come to a swift consensus, at least historically (Fisher 1965). Although inbreeding is generally associated with an increase in homozygosity due to consanguineous matings (Charlesworth and Charlesworth, 1987), its quantification is inherently relative (Wright 1969) and depends on a reference population. This distinction is not merely a matter of definition, as it can extend to differences in biological consequences (Crow and Kimura, 1970; Keller and Waller, 2002). Therefore, it is important to distinguish between the different concepts in which the word "inbreeding" is used (Jacquard 1975). Below, I outline three common definitions of inbreeding (defined in Jacquard (1975) and in Keller and Waller, (2002)).

Inbreeding is often estimated using pedigree information to calculate an individual's inbreeding coefficient $F = \Sigma (1/2)^n (1 + F_{CA})$ (Malécot 1948). F represents a sum of probabilities, n the number of individuals linking an individual's parents through a common ancestor, and F_{CA} the inbreeding coefficient of the common ancestor. Under this definition, an individual's inbreeding level is estimated as being the probability F to possess two homologous alleles that are identical by descent. In other words, this individual is expected to experience a decrease in heterozygosity with probability F, relative to the individuals that form the base population. At the population level, the mean F value is known as Wright's F_{it} (Wright 1951; Wright 1965). Alternatively, inbreeding may result from non-random mating. Here, the reference population consists of randomly mating individuals. Random mating within a given population is expected to yield a certain level of heterozygosity, or Hardy–Weinberg equilibrium. This level of heterozygosity is compared to the observed heterozygosity in the same population, and can be quantified using $F_{is} = 1 - H_o/H_e$. H_o and H_e represent the observed and expected heterozygosities. Finally, inbreeding may be associated with population sub-division and genetic drift. This type of inbreeding is quantified using Wright's F_{st} (Wright 1951; Wright 1965). The three F statistics (F_{it}, F_{is}, and F_{st}) (Wright 1951; Wright 1965) can be associated in the formula: $(1-F_{it}) = (1-F_{is}) (1-F_{st})$.

- Definition of inbreeding depression

A pronounced level of inbreeding can lead to inbreeding depression, with detrimental effects on individual and population viability. These effects may result from two mechanisms: (i) inbred individuals become homozygous at fitness-related loci and experience a fitness decline , or over-dominance. (ii) an increase in homozygosity across the genome exposes recessive, or partially recessive, deleterious alleles and results in detrimental effects on the bearer, or partial-dominance (Charlesworth and Charlesworth, 1987). Furthermore, inbreeding can have profound effects on important evolutionary processes, such as speciation (Lynch 1991) and the evolution of mating systems (Pusey 1987). Finally, from a conservation perspective, inbreeding is as an important factor affecting population dynamics, particularly in relatively small populations (Lacy 1997; Whiteley et al., 2015).

It is, however, important to note that inbreeding does not have universally detrimental effects. Certain animal populations show inconsistent effects of inbreeding, depending on the life-history stage (Mainguy et al., 2009; Hemmings et al., 2012). Moreover, in other studies, even positive associations between inbreeding and fitness have been reported (Richardson et al., 2004; Weiser et al., 2016). These findings highlight the difficulty of predicting effects of inbreeding in natural populations, and the need to adopt integrative approaches that consider different life-history stages and ecological contexts, such as interactions between genetic and environmental variables (Hedrick and Kalinowski, 2000; Keller and Waller, 2002) or parental effects (Crean and Bonduriansky, 2014).

Inbreeding and mate choice

Because of its detrimental effects on fitness, inbreeding is expected to be associated with the evolution of inbreeding avoidance mechanisms (Pusey and Wolf, 1996). However, this does not always occur. Indeed, no evidence of inbreeding avoidance was found in a number of systems known to be negatively affected by inbreeding (Keller and Arcese, 1998; Hansson et al., 2007; Robinson et al., 2012a; Tan et al., 2012). Furthermore, in certain cases, individuals might even prefer to inbreed (Thünken et al., 2007, 2011; Schjørring and Jäger, 2007; Robinson et al., 2012b; Bordogna et al., 2016; O'Brien et al., 2019). A lack of inbreeding avoidance mechanisms in populations that are affected by inbreeding is not always surprising. In fact, even outbreeding can be detrimental, if it results in, for example, the breaking down of co-adapted gene complexes, an increased risk of pathogenic infections, or high costs of venturing into new

environments (Bateson 1983). Thus, under certain conditions, one expects excessive outbreeding to be avoided (Bateson 1982). For example, Bateson (1982) showed in his classic study that female Japanese quail (*Coturnix japonica*) prefer to mate with cousins instead of either siblings or unrelated mates, thus suggesting there is an intermediate, or "optimal", level of outbreeding. Relatedness-based mating strategies may depend on a balance between the costs and benefits of inbreeding (Kokko and ots, 2006), including benefits from an increase in inclusive fitness (Bateson 1983; Lehmann & Perrin, 2003). It is thus challenging to predict whether inbreeding (or outbreeding) will be avoided in a population. Szulkin et al., (2013) summarize the limitations of the theory of biparental inbreeding, with regards to predicting the evolution of inbreeding strategies. These limitations include, for example, basic models that do not consider sex-specific inbreeding avoidance or preference, and do not consider the variance in relatedness within populations. Furthermore, these models tend to ignore other important aspects of mate choice, such as polyandry, and their coevolution with inbreeding strategies. In addition, the authors present a number of fundamental elements to consider in these types of studies. These include a sound statistical approach, using a suitable study system with sufficient variation in relatedness, that is measured reliably for potential and observed mates (Szulkin et al., 2013).

A number of explanations have been proposed for the common inconsistencies in the effects of inbreeding across an organism's ontogeny. Richardson et al., (2004) hypothesised that positive inbreeding effects on survival could arise because a number of inbred, but otherwise healthy, individuals complete embryonic development successfully. These individuals may then have a higher survival probability at later life-history stages than less inbred, but unhealthy, conspecifics. Another potential explanation was proposed by Weiser et al., (2016), who reported a positive association between inbreeding and survival for fledglings, but not juveniles, with inbred mothers in Chatham Island black robins (*Petroica traversi*). This cross-generational interaction of genotypes was attributed to the inheritance of a "proven" genotype by inbred chicks with inbred parents. Finally, offspring of reproductively successful individuals are likely to mate with relatives, thereby compensating for any detrimental effects of inbreeding through higher reproductive output (van Noordwijk & Scharloo, 1981). Regardless of the proximate mechanisms or ultimate functions, the dynamics of biparental inbreeding over an organism's life cycle are exceedingly intricate and necessitate the consideration of several life-stages with different cost/benefit balances between inbreeding and outbreeding.

The sand lizard population of Asketunnan

Sand lizards are relatively small ground dwellers that are distributed over a large area, 8000 x 5000 km (Bischoff 1984). This species is considered vulnerable in Sweden (Gullberg et al., 1999), and has a continuous distribution along the coasts of Halland, Skåne (Scania), Blekinge, and Eastern Småland (Ahlén and Tjernberg, 1992). The rest of the distribution is fragmented, with restricted gene flow between the sub-populations and low genetic variance within them. However, higher levels of genetic variance were observed in central Europe (Figure 1) (Madsen et al., 2000). I studied a population of sand lizards from Asketunnan, on the Swedish West Coast. The genetic variation is low in Asketunnan and the population shows signs of inbreeding depression (Olsson et al., 1996a), as well as effects of genetic relatedness on mate choice (Olsson at al., 1996b; Olsson et al., 2003).

Why sand lizards from Asketunnan?

A number of factors make sand lizards in general, and the Asketunnan population in particular, suitable models to study the effects of inbreeding on survival and population dynamics.

The Asketunnan population is relatively inbred and shows signs of inbreeding depression (Olsson et al., 1996a). In addition, generations overlap, life span sometimes exceeds ten years, and sexual maturation is reached at 2-3 years for males and 2-4 years for females (Olsson 1992). The mating system is polygynandrous and female receptivity asynchronous, resulting in a reduced effective population size (Olsson 1992; Olsson 1994). Thus, inbreeding is likely to occur in the population. In addition, mortality during early ontogeny is high in the population (Corbett and Tamarind, 1979; Olsson et al., 1994), with newly hatched inbred juveniles having a high risk of malformation with a quasi-null probability of surviving (Olsson et al; 1996a). This suggests strong episodes of selection for heterozygosity at this life-stage.

Further to its suitability for exploring inbreeding effects in a free-living population, the Asketunnan population has been studied extensively over decades, with data collected from thousands of individuals, including males, females, and juveniles. Over a period of ten years, gravid females and adult males were captured every spring in the field. They were kept in captivity for egg-laying, and collection of morphological and genetic data. DNA was extracted from thousands of individuals, including juveniles, adults, and unhatched eggs. The DNA was used for paternity analyses and the calculation of heterozygosity indices. Paternity was

successfully assigned for a large a number of juveniles and unhatched embryos, using up to twenty-one microsatellite loci. Newly hatched juveniles were released at random at Asketunnan, of which a number was recaptured over several field seasons. Furthermore, ca. 300 adults were included to analyse restriction fragment length polymorphism (RFLP) of sand lizard MHC (major histocompatibility complex) class 1 genes, resulting in 9 bands available for analyses. Finally, oviparous reptiles are ideal to study the effects of inbreeding during early ontogeny, since infertile, unhatched, eggs, can be easily differentiated from those that did not complete embryonic development. Thus, the ecology and life-history of sand lizards, combined with the accumulated knowledge of the Asketunnan population, make this an ideal system for studying the impact of inbreeding on individual viability, and the resilience of isolated populations with limited gene flow and low genetic variation.

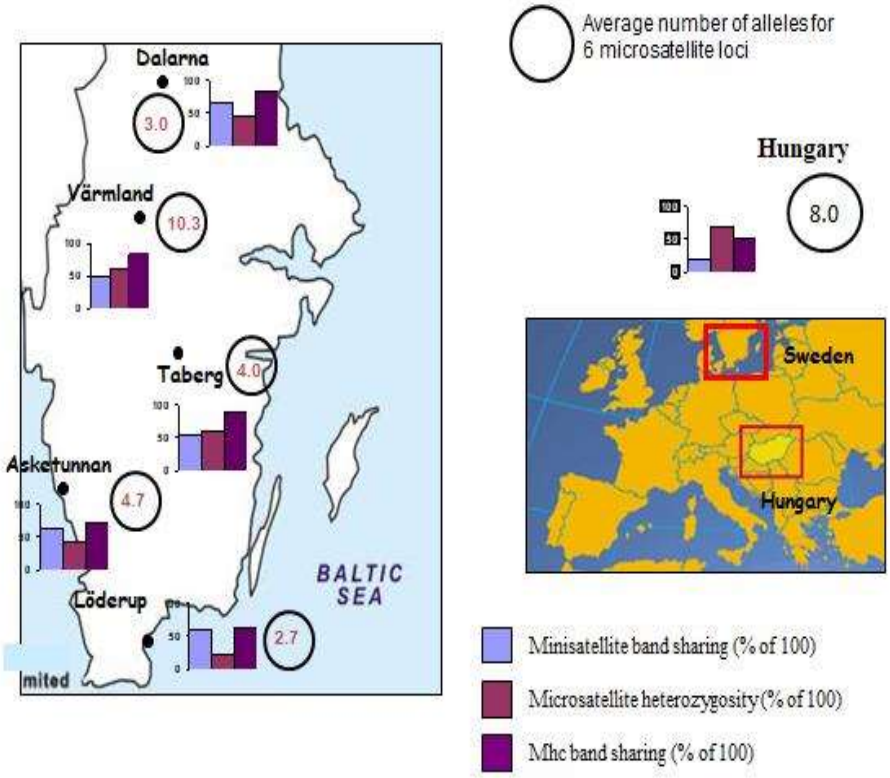

Figure. 1 Comparison of genetic variation in Swedish and central European populations of sand lizards. The types of markers included minisatellites, microsatellites, and MHC bands (From Madsen et al., 2000).

Objectives

The chief objective of this thesis is to contribute to the current knowledge of the effects of inbreeding on viability and the evolution of mating systems in natural animal populations. In a first study (**chapter I**), the fitness effects of consanguineous matings at two early ontogenic stages, embryonic development and first year post-hatching, are measured. In addition, potential interactions with temporal environmental variation and parental effects are examined. Next, the focus is shifted to inbreeding effects on a sexually selected trait, the male badge (**chapter II**). Generally, strongly fitness-related traits are expected to be particularly affected by inbreeding depression (Falconer 1981; Roff 1998; DeRose and Roff, 1999). This is due to strong directional selection and thereby expected erosion of additive genetic variance relative to dominance variance (Roff 1997; Merilä and Sheldon, 1999), combined with the dominance effects associated with inbreeding (Charlesworth and Charlesworth, 1987). Expectedly, numerous reports can be found on the effects of inbreeding on survival and reproductive output (Falconer 1981; Keller and Waller, 2002). However, sexually selected traits have received less attention, with fewer published studies (but see van Oosterhout et al., 2003; Reid et al., 2005; Mariette et al., 2006; Reid 2007; Zajitschek and Brooks, 2010; Marsh et al., 2017; Vega.Trejo et al., 2017). In sand lizards, the male badge size and pigment saturation are intra-sexually selected cues of fighting ability (Olsson 1994) and predictors of mate acquisition (Anderholm et al., 2004), and, hence, relative fitness. Here, the effects of heterozygosity on badge area and saturation are measured, while controlling for important covariates that may affect their expression - year and date of measurement, age/size, and body condition. Two other aspects of badge colouration are also considered, hue and brightness, as being potentially affected by inbreeding. The motivation for the inclusion of additional colour metrics is a more precise understanding of how inbreeding affects both pigmentary and structural components of colour displays. Finally, pairwise phenotypic correlations between colour metrics are measured to explore trade-offs or constraints between the pigmentary and structural components of the display.

By advancing our understanding of inbreeding as a selection agent across ontogeny, **chapter I and II** motivate the study of the mating system in the Asketunnan population, with respect to inbreeding strategy (**chapter III**). In addition, the importance of the MHC as a cue for kin recognition is evaluated. Specifically, the observed patterns of parental relatedness (henceforth referred to as relatedness) are compared to those based on MHC similarity, using simulations based on assumptions of random mating and fertilised eggs as a measure of paternity success.

Relatedness was estimated using a microsatellite-based relatedness coefficient, ”r” (Wang 2002), for observed and potential parental pairs. MHC band sharing was estimated using the band sharing index $D = 2 F_{ab} / (F_a + F_b)$ (Wetton et al., 1987). The simulations included four separate years, between 1998 and 2001. The focus is on MHC assuming that it reflects overall (genome-wide) similarity and, hence, kinship (Potts and Wakeland, 1993; Brown and Eklund, 1994; Penn and Potts, 1999). To test whether potential mate discrimination occurs at the pre- or post-copulatory level, the relative fertilization success of males within mixed-paternity clutches is estimated, based on their differences in the number of MHC bands shared with the female. In summary, **chapter I and II** address ultimate questions related to the impact of inbreeding on viability during early ontogeny, and the expression of the badge at sexual maturity. At the proximate level, **chapter III** is focused on uncovering potential mating patterns, and cues, that promote inbreeding avoidance, tolerance, or even preference.

Up until now, inbreeding depression has been addressed using direct measures of viability and expression of a sexually selected trait - hatching and first year survival, and badge size and colour. In **chapter IV**, the approach shifts to inbreeding effects on cellular function which may be impaired through, for example, effects on metabolic pathways (Kristensen et al., 2006) or cell-mediated immune responses (Reid et al., 2003). Moreover, inbreeding can affect metabolic rates and the ability to allocate energy (Ketola & Kotiaho, 2009). These effects can be exacerbated if more inbred individuals are poor competitors for territories (Meagher et al., 2000), resulting in limited access to food and dietary antioxidants. Thus, the use of a biomarker that reflects somatic stress would be useful to indirectly assess the effects of inbreeding on cellular function. Telomeres may offer this opportunity. These non-coding DNA regions are (largely) located at the ends of chromosomes and assume a protective function during cell division, promoting genomic stability and preventing chromosomes from fusing (Blackburn 1991). The length and attrition of these protective caps are associated with oxidative stress (von Zglinicki 2002; Epel et al., 2004). Thus, with the knowledge that inbreeding disrupts normal cellular function, as well as antioxidant levels, individual-level heterozygosity is predicted to be positively associated with telomere length. In this chapter, long-term data is used (over 1300 offspring, 520 parents, and 10 years of field work) to assess the effects of parental and hatchling heterozygosity on telomere length and attrition.

In the final chapter (**chapter V**), the additive genetic variance and heritability of male badge size are estimated, a fitness-determining trait under intra-sexual selection (Olsson 1994; Anderholm et al., 2004). Sand lizards are sexually dichromatic. During the mating season, the

males develop a lateral area of green nuptial coloration, known as a badge. The badge is displayed towards rivals, and is usually brightly coloured during the early mating season (from late April to June), and fades as the season reaches its end (Olsson 1994). The size and saturation of the badge differ between males (Figure 2), and size varies allometrically with body mass. Olsson (1994) conducted laboratory experiments in which males were manipulated into having larger or smaller badges. The males with enlarged badges were more likely to win contests against males with smaller badges. Additionally, when males had equal badge sizes, the contests lasted longer, indicating that neither male felt "intimidated" and more likely to display sub-dominance (Olsson 1994). Thus, a larger badge is expected to allow larger males, with superior fighting ability, to gain more frequent access to females and increase their reproductive output. So, under this notion, if badge size is under strong directional selection, its additive genetic variance is expected to erode, relative to dominance variance (Roff 1997; Merilä and Sheldon, 1999), hence the "lek paradox" (Kirkpatrick and Ryan, 1991; Andersson 1994; Rowe and Houle, 1996). However, the assumption that sexually selected, and life-history, traits will have low additive genetic variance, due to directional selection, is not always realized (Houle 1992; Pomiankowski and Moller, 1995; Rowe and Houle, 1996). This confronts the lek paradox, highlighting the potential significance of environmental variation and condition in the expression of sexually selected traits (Rowe and Houle, 1996; Griffith et al., 1999; but see Cotton et al., 2004 and Van Homrigh et al., 2007), and the maintenance of additive genetic variance under seemingly strong directional selection. The rationale of this "genic capture" is that condition-dependent traits capture genetic variance in condition (Rowe and Houle, 1996), assuming a polygenic underpinning of condition. In this chapter, predictions of sexual selection theory are tested with respect to the erosion/maintenance of additive genetic variance in sexually selected traits, and the role of condition dependence in maintaining such variance. Specifically, using animal models, the additive genetic variance in badge size, and in body condition, are estimated for adult male sand lizards. Finally, the maternal and permanent environmental effects on badge size and body condition are quantified.

Figure. 2 The badge is located on the lateral area of a male's body, between the front ant the hind leg . The images show three males with badges that differ in size, from small to large (top to bottom image) (photography courtesy of Willow Lindsay).

Methods

This section describes the most relevant theoretical and methodological aspects applied in the thesis to facilitate the reader's conception of the different chapters. For detailed descriptions of particular methods, the reader is encouraged to visit relevant chapters.

Estimating inbreeding

Pedigrees are commonly used to estimate individual-level inbreeding. However, in the absence of such information, marker-based heterozygosity metrics are often used as substitutes. In fact, these metrics can even outperform pedigree-based methods in predicting the realized portion of a genome that is identical by descent (Hemmimgs et al., 2012; Kardos et al., 2015). These findings reveal that marker-based heterozygosity metrics show promising prospects to estimate inbreeding, particularly in natural populations in which parentage is exceedingly difficult to assign. Accurate pedigrees are both difficult and costly to obtain

(Keller et al., 2011). Their use is further limited by the depth of the available data (Stam 1980) and the lack of precision of pedigree-based inbreeding coefficients, as they represent approximations of the degree of genomic autozygosity (Franklin 1977; Hill and Weir, 2011), instead of realized heterozygosity.

Below, are defined four commonly used metrics of individual-level inbreeding, based on multi-locus heterozygosity.

d^2 : Developed by Coulson et al., (1998) and uses information of coalescence time for microsatellites. Specifically, it is a measure of the squared difference in the number of repeats between two microsatellite alleles at a given locus. d^2 was originally developed to allow researchers to differentiate between high and moderate levels of inbreeding. However, its use was questioned both empirically and theoretically, based on weak correlations with inbreeding and its fitness consequences (Hedrick et al., 2001; Tsitrone et al., 2001; Goudet and Keller, 2002).

SH (standardized individual heterozygosity): Developed by Coltman et al., (1999), this index uses observed heterozygosity as the normalization factor, given by the formula *SH* = (proportion of heterozygous typed loci/mean heterozygosity of typed loci), with a range between 0 and 2. Although it is prefered to a straightforward estimate of heterozygosity, *SH* does not consider the differences in allele frequencies between loci and is based on a linear relationship between the heterozygosity at a given locus and its number of alleles. This ignores situations in which the relationship is, for example, exponential, with the effects of highly variable loci weighing more than less variable ones (Aparicio et al., 2006).

IR (Internal relatedness): The name “internal relatedness” derives from the fact that the index involves parental half genotypes. This method is based on allele sharing, with more or less weight given to alleles depending on their degree of commonness. *IR* is calculated as IR = (2H – $\sum f_i$) / (2N - $\sum f_i$). Here, H represents the number of homozygous loci, N the number of loci, and $\sum f_i$ the sum of the frequencies of the alleles included in a genotype. *IR* ranges between -1 and 1, with increasing heterozygosity towards negative values and increasing homozygosity towards positive ones (Amos et al., 2001). The distribution of *IR* values is asymmetrical, leading to potential issues when estimating individual-level heterozygosity. For example, completely homozygous genotypes always generate an *IR* value of 1, irrespective of allele frequencies. Conversely, a value of -1, or 100% heterozygosity, can only occur when a genotype consists entirely of loci with two alleles. Furthermore, the effect of rare alleles on *IR* is not uniform, and

depends on whether the locus is homozygous or heterozygous. Finally, the effects of the asymmetrical distribution of *IR* could be exacerbated in populations subject to immigration (Aparicio et al., 2006).

HL (homozygosity by loci): *HL* estimates homozygosity/heterozygosity weighed by locus, instead of alleles, based on allelic variability. Individual *HL* is given by the formula $HL = \sum E_H / \sum E_H + \sum E_j$

where $\sum E_H$ is the sum of the expected heterozygosities of the observed homozygous loci and $\sum E_j$ the sum of the expected heterozygosities of the observed heterozygous loci. *HL* ranges between 0 and 1, with 0 indicating complete heterozygosity and 1 complete homozygosity (Aparicio et al., 2006).

How was individual inbreeding estimated?

Three indices (*SH*, *IR*, and *HL*) were calculated to estimate individual-level heterozygosity, as a proxy for inbreeding, using 21 microsatellites for 3967 individuals (Olsson et al., 2011) in the R package Rhh (Alho et al., 2010). In addition, the pairwise correlations between the three indices were estimated. All correlations were strong and statistically significant (Figure 3).

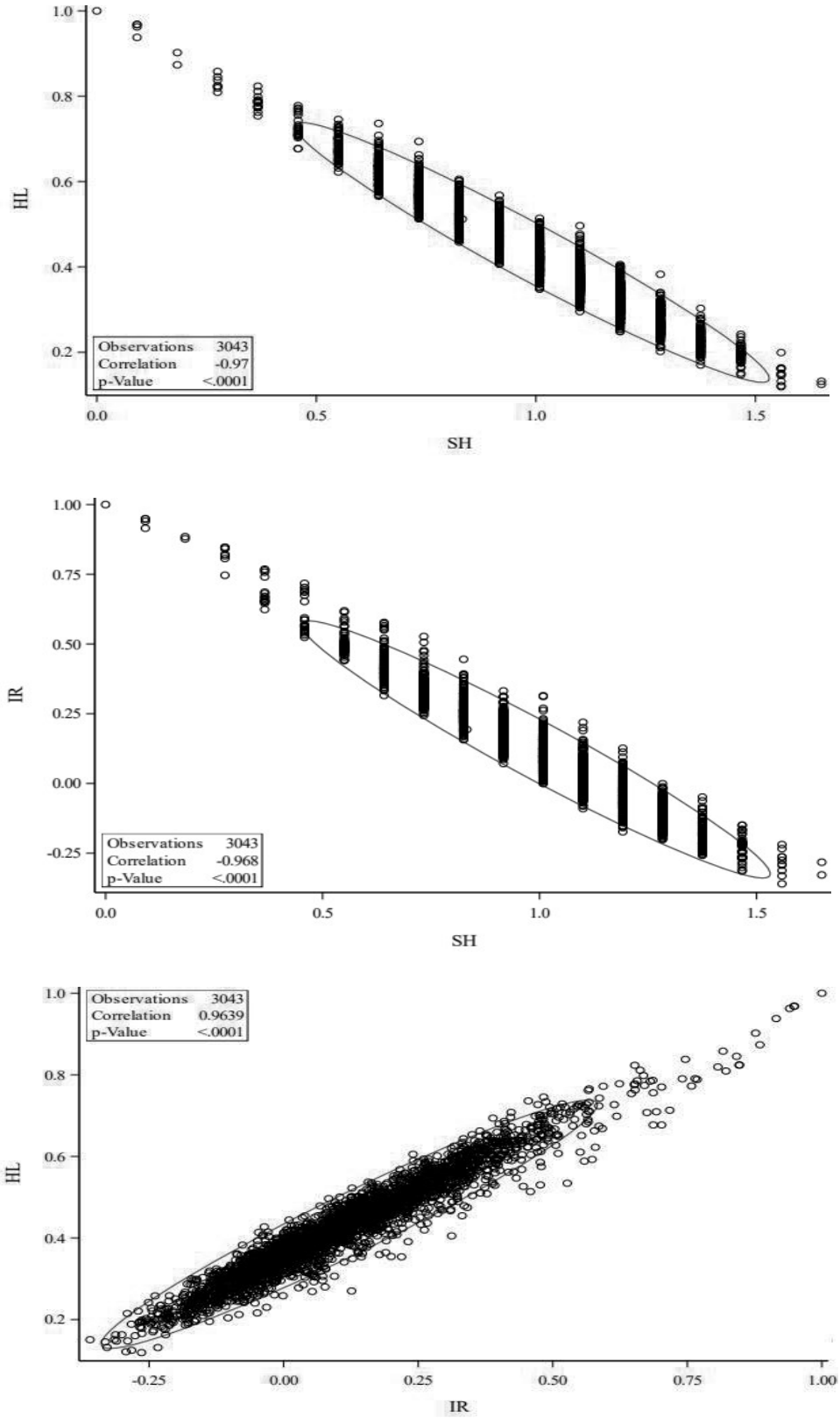

Figure. 3 Pairwise correlations between the three indices (*SH*, *IR*, and *HL*) with 95% prediction ellipses, assuming bivariate normal distributions.

Detection of inbreeding in the study population

The strength of heterozygosity-fitness correlations (HFC) depends on identity disequilibrium (ID) between loci (Szulkin et al., 2010), or correlations of homozygosity/heterozygosity due to variance in individual inbreeding (Weir and Cockerham, 1973). Rhh was used to estimate ID, or the inbreeding signal captured by the indices of multilocus-heterozygosity (*SH*, *IR*, and *HL*), by calculating a heterozygosity-heterozygosity correlation (HHC) (Balloux et al., 2004). Thus, the loci were randomly divided into two groups, and the heterozygosity indices were calculated separately for each group. The correlation between the two groups was then estimated, yielding the HHC. The procedure was repeated 1000 times. All HHCs were positive (0.213 for IR, 0.170 for *HL*, and 0.141 for *SH*) with no confidence interval including 0. Thus, the loci used in the analyses reveal ID and a signature of inbreeding in the study population. However, the inbreeding signal was relatively weak. This could have resulted from the use of a limited number of 21 microsatellite loci. The signal is nonetheless detectable and significant, likely due to the small size of the Asketunnan population and its high potential for inbreeding (Olsson et al., 1996a).

The power of HHC to detect ID is debatable, due to, for example, complicated distributions of non-independent coefficients (Szulkin et al., 2010). Therefore, the parameter *g*2 was also calculated to detect ID. *g*2 is central to HFC theory and integrates information from all loci to produce a single value. In addition, this parameter remains constant irrespective of the source of inbreeding, or the selected loci. Finally, *g*2 varies according to population-specific inbreeding, mean and variance (David et al., 2007; Szulkin et al., 2010). Positive g2 values indicate variance in inbreeding, and thus grounds for HFC to occur. *g*2 was positive and statistically significant in the study population.

Statistical analyses

The fundamental statistical concepts applied in the thesis are outlined in this section.

All statistical analyses were performed in SAS (Statistical Analysis System) 9.4 and ASReml-W release 4.1.

- Multilevel models

Rooted in sociology in the 1950s and early 1960s (Merton, 1957), and education in the 1970s (Barr and Dreeben, 1977; Block and Burns, 1976; Bronfenbrenner, 1976), this type of statistical model considers hierarchical structuring within populations, such as families, neighbourhoods, or communities. Individuals are often nested under group level variables, which can influence their behaviour (Merton, 1957; Blalock 1984). Failing to account for contextual effects may violate the assumption of observation independence, resulting in significant intraclass correlation coefficients (ICC) and sizeable Type-1 errors. Thus, contemporary multilevel modelling offers an appropriate analytical structure for analysing hierarchically structured data sets, as it considers both individual and group-level effects (Wang et al., 2011). In the following sections, I describe the general structure and inference approaches used in multilevel modelling. In particular, I focus on its application to binomial error distributions, demonstrating the effectiveness of this type of statistical procedure for the analyses performed in my research (e.g. binary survivorship data) (see Wang et al., (2011) for a detailed description of multilevel modelling).

- Steps for fitting a multilevel model

Usually, the first step in multilevel modelling is to estimate the intraclass correlation coefficient (ICC).

ICC is calculated using the formula: $ICC = \sigma^2_b / \sigma^2_b + \sigma^2_w$ (1)

here the nominator represents the variance between groups (σ^2_b) and the denominator the total variance. σ^2_w represents the within-group variance (Shrout & Fleiss, 1979). So, to calculate ICC, σ^2_w and σ^2_b must be estimated. This is done using an empty model:

$Y_{ij} = \beta_{0j} + e_{ij}$ (2) or a random intercept model, comparable to an ANOVA (one-way analysis of variance).

In equation (2), y_{ij} represents the outcome of individual i in group j. β_{0j} represents the mean outcome of group j and e_{ij} the within-group variance, or σ^2_w.

The intercept variation due to the between-group variance is shown in the following equation:

$\beta_{0j} = \gamma_{00} + \upsilon_{0j}$ (3)

where γ_{00} is the overall mean of Y_{ij}, and υ_{0j} the between-group variance, or σ^2_b.

Combining equation (2) and (3) gives $Y_{ij} = \gamma_{00} + \upsilon_{0j} + e_{ij}$ (4)

From equation (4) $\text{var}(Y_{ij}) = \text{var}(\upsilon_{0j}) + \text{var}(e_{ij})$

$= \sigma^2_b + \sigma^2_w$

ICC can then be calculated using equation (1). Multiple modelling is to be considered for any statistically significant ICC.

- Adding level 1 and level 2 explanatory variables

After ICC estimation, individual (level 1) or group-level (level 2) explanatory variables may be added to the model (Hox 1995; Singer 1998). Below, I exemplify how a group-level explanatory variable is added (w_{1j}):

By adding the fixed effect w_{1j} to equation (3), we obtain:

$\beta_{0j} = \gamma_{00} + \gamma_{01} w_{1j} + \upsilon_{0j}$ (5)

Combining equations (2) and (5) gives:

$Y_{ij} = \gamma_{00} + \gamma_{01} w_{1j} + (\upsilon_{0j} + e_{ij})$ (6)

Equation (6) includes two random effects ($\upsilon_{0j} + e_{ij}$), and the fixed effect (γ_{01}).

- Assessing cross-level interactions

Up until now, I have only considered the intercept and residuals as random variance components. Multilevel models can also accommodate random level-1 slope coefficients, as shown in the following equations:

$Y_{ij} = \beta_{0j} + \beta_{1j} z_{1ij} + e_{ij}$ (7) Shows a model with a level 1 explanatory variable (z_{1ij}).

$\beta_{0j} = \gamma_{00} + \gamma_{01} w_{1j} + \upsilon_{0j}$ (5) A level 2 explanatory variable is added (w_{1j}).

$\beta_{1j} = \gamma_{10} + \gamma_{11} w_{1j} + \upsilon_{1j}$ (8) This model has the fixed effect γ_{11}, the random term υ_{1j}, and the level 2 explanatory variable (w_{1j}), hence the interaction between z_{1ij} and w_{1j}.

The complete model is as follows:

$Y_{ij} = \gamma_{00} + \gamma_{01} w_{1j} + \gamma_{10} z_{1ij} + \gamma_{11} w_{1j} z_{1ij} + (\upsilon_{0j} + z_{1ij} \upsilon_{1j} + e_{ij})$ (9)

- Application of multilevel modelling to discrete outcome measures

Discrete outcome measures (such as yes or no) are accommodated by generalized linear mixed models, or extensions of linear mixed models. Generalized linear mixed models can be applied to different types of discrete outcomes, including ordinal, nominal, count, and binary. Here, I focus on binary outcomes, by providing a brief description of fixed-effects logistic regression models (a type of generalized linear models) and their extension to mixed-effects logistic regression models (a type of generalized linear mixed models).

- Fixed-effects logistic regression models

The link function, or *logit*, in a logistic regression model is formulated as:

$\text{logit}(p) = \log(p / p - 1) = \beta_0 + \sum_{k=1}^{K} \beta k\, xk$

Here, each explanatory variable x is associated with its regression slope coefficient β. The logit allows the transformation of non-linear relationships between covariates and the outcome into linear ones. *P,* the probability of an event occurring, represents the mean of the outcome measure (y), with a variance var (y) = $p(1 - p)$.

From the logit model, the probability of occurrence of an event:

$P = \Pr(y_i = 1|X) = \exp(z) / 1 + \exp(z)$ with $z = \beta_0 + \sum_{k=1}^{K} \beta k\, xk$

This logistic function has an S-shaped distribution, rather than a linear one - as in the relationship between the *logit* (or log-odds) and the covariates.

- Mixed-effects logistic regression models

Mixed-effects logistic regression models differ from logistic regression models, by accounting for ICC. Here, the model has both fixed and random effects, and is formulated in matrix format as:

$\text{Log}(p / 1 - p) = X\beta + ZU$

X and Z represent the design matrices associated with the fixed effect parameter (vector β) and the random effect parameter (vector *U*).

Similarly to linear multilevel models, multilevel logistic regression models can be expressed as:

$\text{Log}(p_{ij} / 1 - p_{ij}) = \beta_{oj} + \beta_{1j} x_{1ij}$

Where the intercept (β_{0j}) and the slope (β_{1j}) are random, representing linear functions of level 2 predictors. Note that, unlike linear multilevel models, this model has no error term.

- Concluding remarks

By accounting for ICC and observation dependence, multilevel models decrease the risk of Type-1 error, and thus provide a reliable statistical framework to analyse complex data sets with observations nested over one or several levels. These hierarchical models are applicable to both linear and generalized linear models, including data from a large range of distributions. The use of multilevel modelling is essential to my research, which involves observations of, for example, juvenile sand lizards that may share one or both parents. This type of crossed random effect is accommodated by a number of procedures developed in SAS. These include, for example, *PROC MIXED* to model continuous outcome measures, or *PROC NLMIXED* and *PROC GLIMMIX* which are suited to categorical and count data, such as Poisson, ordered logit, multinomial logit, multilevel logit, and probit (Wang et al., 2011).

- Animal models

Animal models are based on similar principles as multilevel models, by including fixed and random effects. They are typically used to estimate the contribution of different sources of variance to phenotypic expression (Lynch and Walsh, 1998). This form of mixed models has been used extensively in plant and animal breeding programs (Henderson 1950, 1975, 1984) but, surprisingly, not in evolutionary biology until decades after they were developed (Konigsberg and Cheverud, 1992; Cheverud and Dittus, 1992; Knot et al., 1995; Réale et al., 1999; Kruuk et al., 2000; Milner et al., 2000).

The general form of an animal model is: $y_i = \mu + a_i + e_i$

where y_i is the phenotypic value of individual *i*. μ represents the population mean, and e_i the residual of individual *i*. a_i represents the additive genetic value of individual *i*. σ^2_A is the variance associated with the random effect (a_i), with a mean of 0. This variance component is the additive genetic variance to be estimated. The residual variance σ^2_R also has a mean of 0. Thus, the total phenotypic variance for the above model is $\sigma^2_A + \sigma^2_R$. The heritability of trait *y* is given by: $h^2 = \sigma^2_A / (\sigma^2_A + \sigma^2_R)$, and represents the fraction of the total phenotypic variance that is due to additive genetic effects. The model can be extended by adding fixed effects, such as age, year, or sex. More random effects can also be added, such as maternal or permanent environmental effects. The additive genetic effect, or breeding value, of each individual is estimated by

considering its phenotypic value and those of its relatives in the population, using pedigree information to construct an additive genetic relationship matrix (Kruuk 2004).

The animal models that I used were fitted in ASReml-W release 4.1.

Main results

Detailed results can be found in the thesis' chapters. Below, I summarize the most relevant results relating to the main objectives of the thesis.

Chapter I

The main question addressed in this chapter was the effect of individual-level standardized heterozygosity (SH), as a proxy for inbreeding, on hatching success and first year survival, for embryos and juvenile sand lizards.

SH was a significant predictor of hatching success, with an odds ratio of 1.29 (based on a 10% increase from the mean SH in the data) (figure 4) but did not affect first year survival (figure 5), and did not interact significantly with year in either model. Year significantly predicted both hatching success and first year survival. Finally, juvenile sex did not affect first year survival. Sex-specific effects on hatching probability were not considered, as unhatched embryos could not be sexed.

Both paternal and maternal identity had an effect on hatching success, but not on first year survival. The intraclass correlation coefficient (ICC) for maternal ID was relatively large (0.70), while the ICC for paternal ID was substantially smaller (0.25).

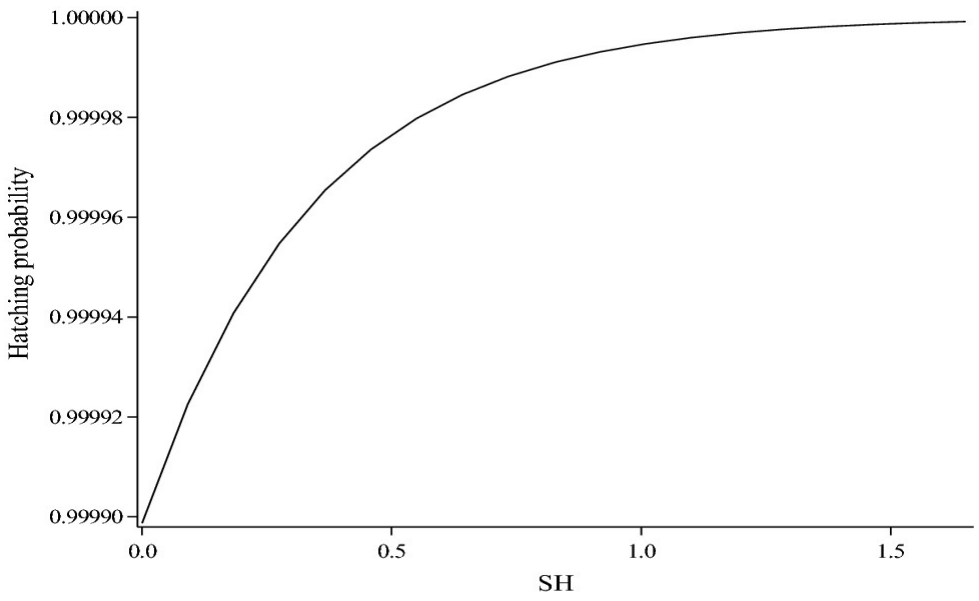

Figure. 4 Effect of embryo, or juvenile, individual-level standardized heterozygosity (SH) on hatching probability.

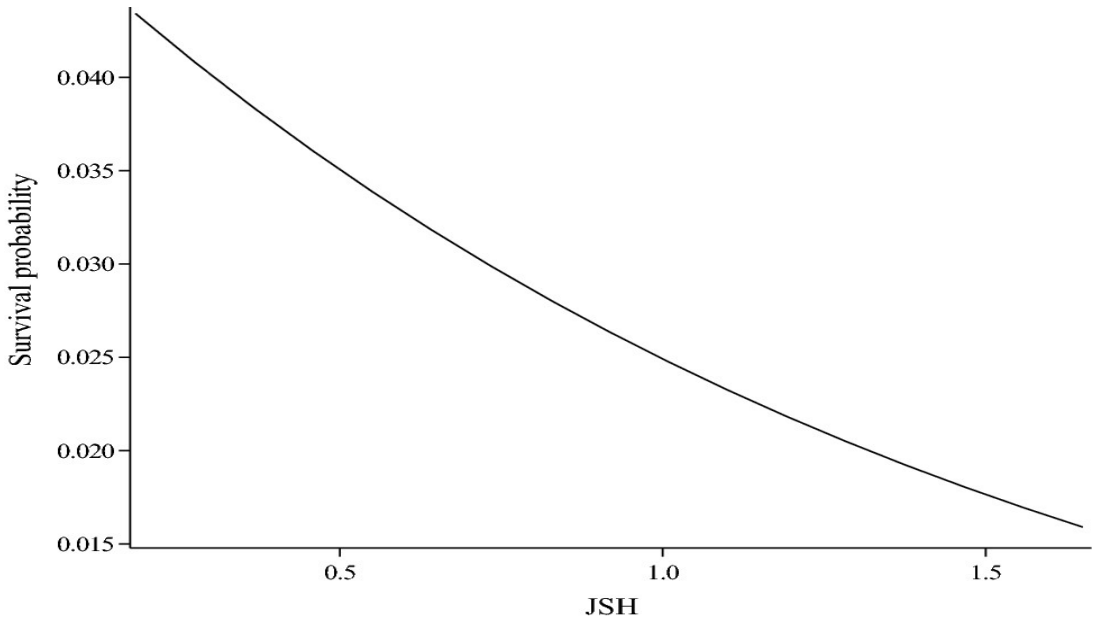

Figure. 5 Effect of juvenile individual-level standardized heterozygosity (JSH) on the probability of surviving the first year post-hatching.

Chapter II

In this chapter, the main objective was to measure the effect of inbreeding on badge size and colour, using SH as a proxy for genome-wide heterozygosity. SH had a positive and statistically significant effect on badge size, saturation, and brightness, but not hue. The effect sizes (R^2), i.e. the proportion of variance in badge size and colour explained by individual-level heterozygosity, were small, but somewhat larger for hue (0.038% for badge size, 0.8% for saturation, 0% for hue, and 1.3% for brightness). Body condition significantly affected badge size and saturation, but not hue or brightness. Finally, badge size, hue, and saturation correlated significantly, whereas brightness did not correlate with any badge component (Table 1). This finding was supported by a principal component analyses (PCA) that revealed the existence of two components that jointly explained 67.3% of the variance in the data. Badge size, hue, and saturation showed relatively high loadings on principal component one (PC1), while brightness had a relatively high loading on principal component 2 (PC2) (Figure 6).

Table.1 The table shows the pairwise correlations between badge size, hue, saturation, and brightness. For each variable, are shown the Spearman correlation coefficient (top) and *p*-value (bottom). The statistically significant *p*-value are bolded.

	Size	Brightness	Hue	Saturation
Size	-	0.05600	0.13039	0.38736
		0.3403	**0.0259**	**<.0001**
Brightness	0.05600	-	-0.03284	-0.03066
	0.3403		0.5763	0.6018
Hue	0.13039	-0.03284	-	0.45256
	0.0259	0.5763		**<.0001**
Saturation	0.38736	-0.03066	0.45256	-
	<.0001	0.6018	**<.0001**	

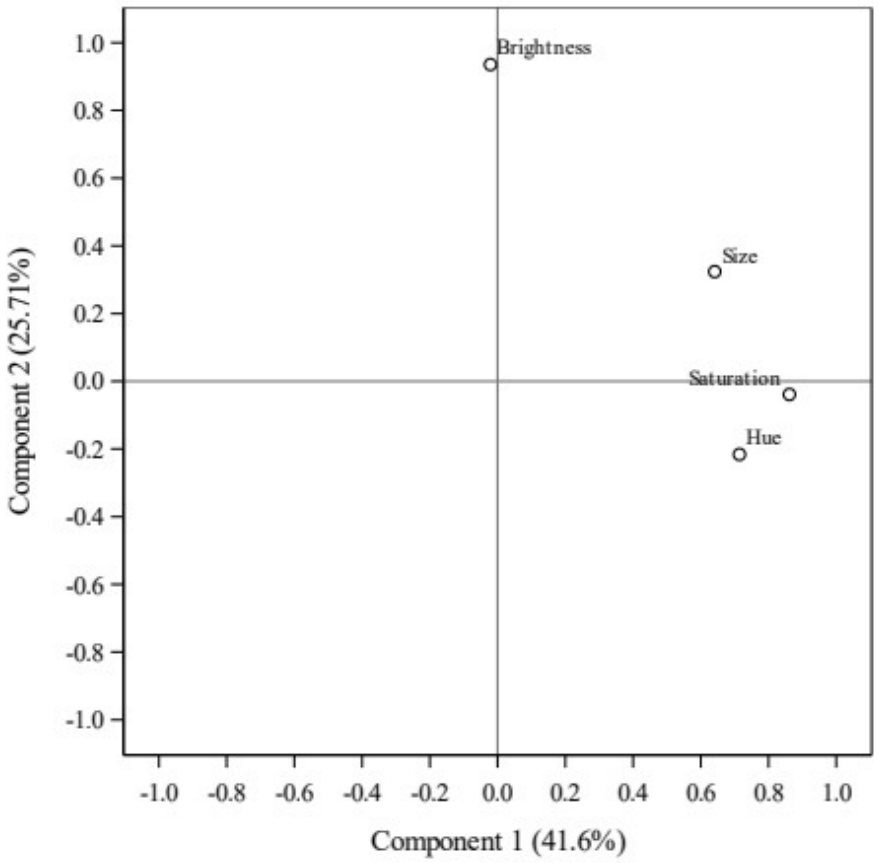

Figure. 6 Principal component analyses of the four badge size and colour elements. Badge size, hue, and saturation had high loadings on component 1, while hue had a relatively high loading on component 2. The proportion of explained variance by each component is shown between brackets.

Chapter III

This chapter is focused on assessing the effect of relatedness on paternity success, and the role of the MHC as a cue for inbreeding avoidance.

Paternity success (estimated using fertilised eggs) was not random with respect to MHC band sharing (figure 7), but it was random with respect to relatedness (figure 8). The successful parental pairs shared less MHC bands than they would have under random mating. This was not the case when relatedness was considered (estimated using a microsatellite-based relatedness coefficient, r). Furthermore, the correlation between the two indices, D and r, was not statistically significant. Finally, in mixed- paternity clutches, no effect of relative number of shared bands (between a successful male and the female) on fertilization success was found (Figure 9), suggesting that the MHC disassortative mating pattern derived from pre- rather than post-copulatory mechanisms (such as cryptic female choice).

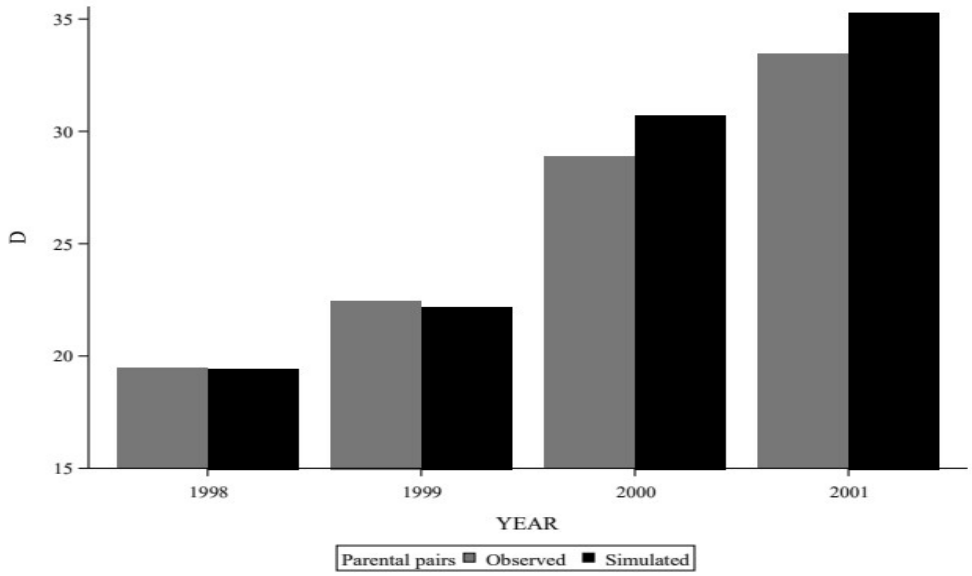

Figure. 7 Difference between the sum of D's of the observed parental pairs and the mean sum of D's of the expected parental pairs. The mean sum of the expected parental pairs was obtained from simulations based on the assumption of random mating in the study population. The simulations included 4 years, between 1998 and 2001.

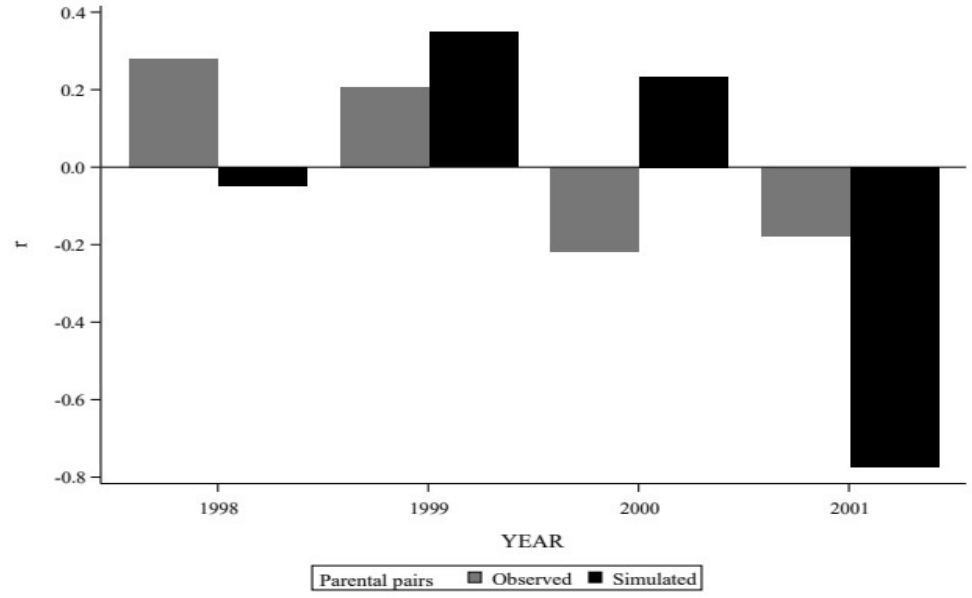

Figure. 8 Difference between the sum of r's of the observed parental pairs and the mean sum of r's of the expected parental pairs. The mean sum of the expected parental pairs was obtained from simulations based on the assumption of random mating in the study population. The simulations included 4 years, between 1998 and 2001.

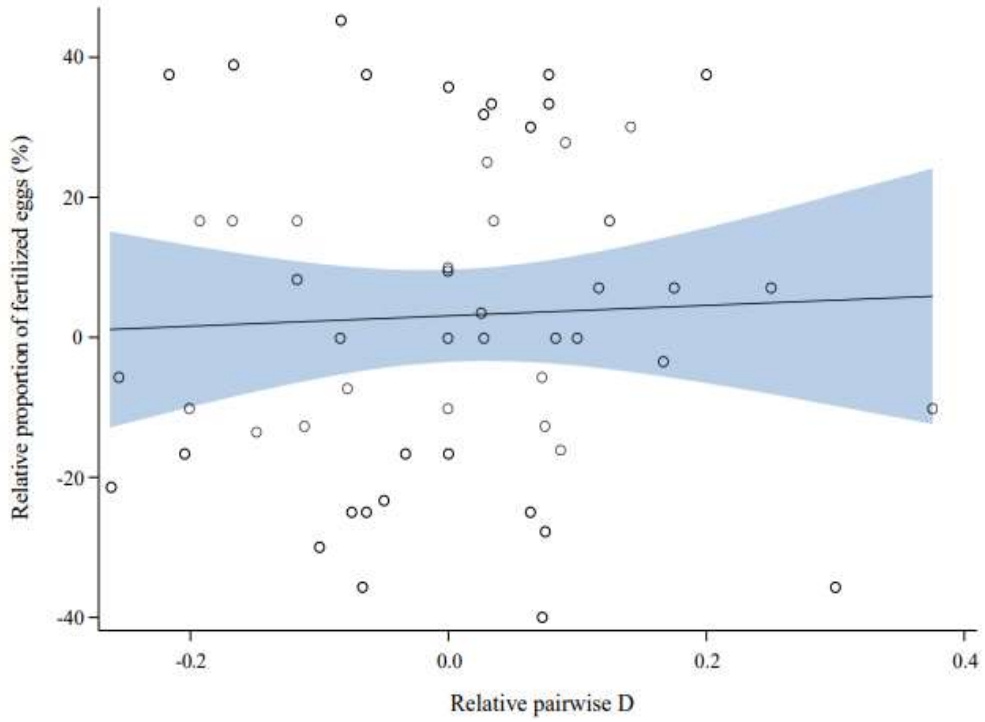

Figure. 9 Effect of relative number of shared bands (relative pairwise D) on relative proportion of fertilized eggs in mixed-paternity clutches. The line shows the predicted mean and the shaded area the 95% confidence limits. Each circle represents one randomly selected male for each of 56 mixed-paternity clutches.

Chapter IV

Paternal and maternal identities (IDs), and their interaction, significantly affected hatchling telomere length, explaining approximately half of the variance in the data. A smaller fraction of the variance was significantly explained by hatchling sex and average seasonal temperature (3.1% and 4.9% respectively). Higher temperatures were associated with longer telomeres, while female hatchlings had shorter telomeres than males. The only effect attributable to inbreeding was that deviations in maternal heterozygosity levels (from mean female heterozygosity) were associated with shorter hatchling telomeres, which may suggest stabilizing selection. This effect was however only detectable while controlling for paternal ID. Hatchling heterozygosity had no significant effect on telomere length, except when parental IDs, and their interaction, were removed from the model.

Chapter V

The impetus for the final chapter was to estimate the relative contributions of genes and environment to the expression of the badge. Specifically, the additive genetic variance and heritability of badge size were estimated, while considering maternal and permanent environmental effects. In addition, the importance of genic capture (Rowe and Houle, 1996) for maintaining genetic variation for a trait under sexual selection was evaluated. This was done by estimating the additive genetic variance, and heritability, of body condition.

A statistically significant level of additive genetic variance in badge size was observed, with a heritability of 25%. This effect was obtained in an animal model containing individual ID as only random effect, as maternal effects were statistically non-significant and removed from the model. The model also included a number of fixed effects - year and date of sampling, snout-vent length (as a proxy for age) and body condition. The additive genetic variance in body condition was relatively low and statistically non-significant. Conversely, a male's permanent environment significantly affected body condition.

Summary and conclusions

The impact of inbreeding on fitness can vary across life-history stages in sand lizards, with an inconsistent selection pressure on heterozygosity between incubation and first year post-hatching. Thus, it is essential to consider the effects of inbreeding across ontogeny, as well as parental and environmental effects, in both theoretical and empirical studies, aimed at the management and conservation of small populations at risk of inbreeding depression.

Individual-level heterozygosity significantly predicted both badge size and colour, a sexually selected trait. The effect was, however, relatively weak, but somewhat stronger for badge brightness, relative to badge size or saturation, and non-significant for hue. This finding raises questions and encourages experimentation to establish the role of structural coloration in conveying information between rivals, using sexually selected colour signals. Body condition significantly predicted badge size and saturation, but not hue or brightness. Moreover, the pairwise phenotypic correlations between badge size, hue, and saturation were significant, whereas brightness did not correlate with any other badge feature. This suggests differences in selection pressure, and evolutionary trajectories, for the multiple components that form the badge. Multivariate animal models offer a promising means to explore the genetic covariances between the badge pigmentary and structural components, as well as establishing the relative importance of external sources of variation, such as maternal and environmental effects.

In spite of clear detrimental effects of inbreeding on fitness-determining traits in the Asketunnan population, no evidence of disassortative mating according to genome-wide similarity was found, using fertilized eggs as a proxy for mating success. Fertilised eggs represent the outcome of a complete copulatory process, or pre- and post-copulation. In previous work on the same population, evidence of biased paternity by females, in favour of more genetically dissimilar males, was found (Olsson at al., 1996b). These findings demonstrate the importance of considering both pre- and post-copulatory stages separately when studying mate choice in natural populations. Evidence of a disassortative mating pattern according to MHC similarity was found, also using fertilized eggs, in spite of a lack of evidence of inbreeding avoidance. Moreover, the correlation between relatedness and MHC band sharing was non-significant. These findings contradict the notion that parental pair similarity at the MHC predicts genome-wide similarity, in the study population. Therefore, I recommend a cautious approach when inferring genome-wide similarity using specific genomic regions, such as loci responsible for immunocompetence. No biased paternity towards more MHC-dissimilar males was found in mixed-paternity clutches. This presents

indirect evidence that the MHC-based disassortative pattern of parental pairs that was observed likely occurred pre-copulation, consistent with previous field observations where both preference and association between potential mates occurred in favour of MHC dissimilarity (Olsson et al., 2003). Thus, the occurrence of mate or sperm selection in the Asketunnan population appears to depend on the type of selection pressure, immunocompetence or inbreeding.

One of the main aims of the thesis was to measure the effect of inbreeding on telomere length and attrition, as an indirect assessment of cellular function (Reid et al., 2003; Kristensen et al., 2006). Surprisingly, only minor effects of individual-level heterozygosity on telomere length were found. Neither parental or hatchling standardized heterozygosity (SH) significantly affected hatchling telomere length. This finding could be attributed to the ideal incubation conditions, that may have buffered any inbreeding effects during embryonic development. Alternatively, a transgenerational inbreeding effect may have been incorrectly attributed to other types of parental effects, resulting in the underestimation of a "true" inbreeding depression. Nevertheless, the fact that no effect of inbreeding on telomere length was found warrants future research, in which other potentially important confounding sources of variation are considered, such as parental effects. Furthermore, combining sequencing, transcriptomic and methylation analysis may prove fruitful in increasing our resolution to detect inbreeding effects on telomere attrition, instead of relying on a limited number of microsatellites to estimate genome-wide heterozygosity. Both parental IDs and their interaction had a significant effect on hatchling telomere length (explaining approximately 50% of the total variance in the data). Ambient spring temperature and juvenile sex significantly predicted hatchling telomere length, with male hatchlings having longer telomeres than females. Finally, unlike intrinsic measures of maternal SH, deviation from the mean maternal heterozygosity resulted in shorter hatchling telomeres. Thus, this trans-generational effect of heterozygosity on telomere length appears to be under stabilizing selection. This study adds to a growing body of literature on the effects of inbreeding on telomere dynamics (Delany et al., 2000; Sridevi et al., 2002; Garcia-Cisneros et al., 2015; Bebbington et al., 2016), and provides a sound basis for gaining deeper insights into the genetic and epigenetic patterns underlying telomere attrition, or elongation.

The final objective of the thesis was to estimate the additive genetic variance in badge size and body condition, and hence their heritabilities. A significant amount of additive genetic variance in badge size was found, in spite of the trait being under directional selection through

male-male competition (Olsson 1994). This finding cannot be explained by genic capture (Rowe and Houle, 1996), because of the low, and statistically non-significant, estimate of additive genetic variance in body condition that was found. Instead, body condition was significantly influenced by the permanent environment experienced by a male. Finally, maternal effects were not significant predictors of badge size, or body condition. This suggests that the multifaceted genetic architecture of the badge (underlying pigmentary and structural aspects of colouration), combined with sex (Singh and Punzalan 2018; Cally et al., 2019) and age-specific (Olsson 1994) selection, may (at least partly) explain the maintenance of additive genetic variance in this fitness-determining trait.

Acknowledgments

First and foremost, I want to thank my supervisor, Mats Olsson, without whom I would not be writing this text today. I will always be thankful for Mats' help, and the fantastic intellect and wisdom that he is always willing to share.

Thank you, my family and friends for being so supportive, particularly my mother for encouraging me to go to University and always believing. Thanks to my partner for her patience ,support, and love. I want to thank my wonderful daughter for her love, and simply for being here.

Thank you!

Erik Wapstra, for co-supervising the thesis, and his valuable input and suggestions, both general and science-related. I also want to thank Erik for the dozens of delicious pizzas that he only can make in record time.

Erik Svensson, for co-supervising the thesis, and his invitation to the Christmas conference, to meet the ecologists from Lund University.

My, other, co-authors, for their tremendous work and feedback: Tonia Schwartz, Beata Ujvari, Erik Wapstra, Willow Lindsay, and Pierre Duchesne.

Willow Lindsay, for her enormous engagement in the manuscripts, and the precious scientific input that made the journey a lot easier.

Donald Blomqvist, for his valuable help with statistics, and for sharing his vast knowledge.

Karin Hårding, for her support and encouragements.

My examiners, Bengt Gunnarsson and Staffan Andersson for following my progress, and providing useful comments on the thesis.

The fantastic and highly efficient administrative staff of the University of Gothenburg. I particularly want to thank Lilioth Olsson, Ingela Lyck and Olinda Gonzales for their kindness.

Magnus Lovén Wallerius, for answering the hundreds of questions that I asked him through the years, and being such a great colleague and friend.

Eva-Lotta Blom, for sharing her office with me, and the great tips and suggestions that she provided. I am extremely thankful for her help, especially when I started my PhD studies.

Henrik Imberg, for his help with statistics, digging beyond the available statistical methods to provide solutions.

My fellow PhD students for the amazing atmosphere that they create at the department.

Financial support was provided by the Australian Research Council, the Swedish Science Council, Herbert & Karin Jacobssons stiftelse, and the University of Gothenburg.

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