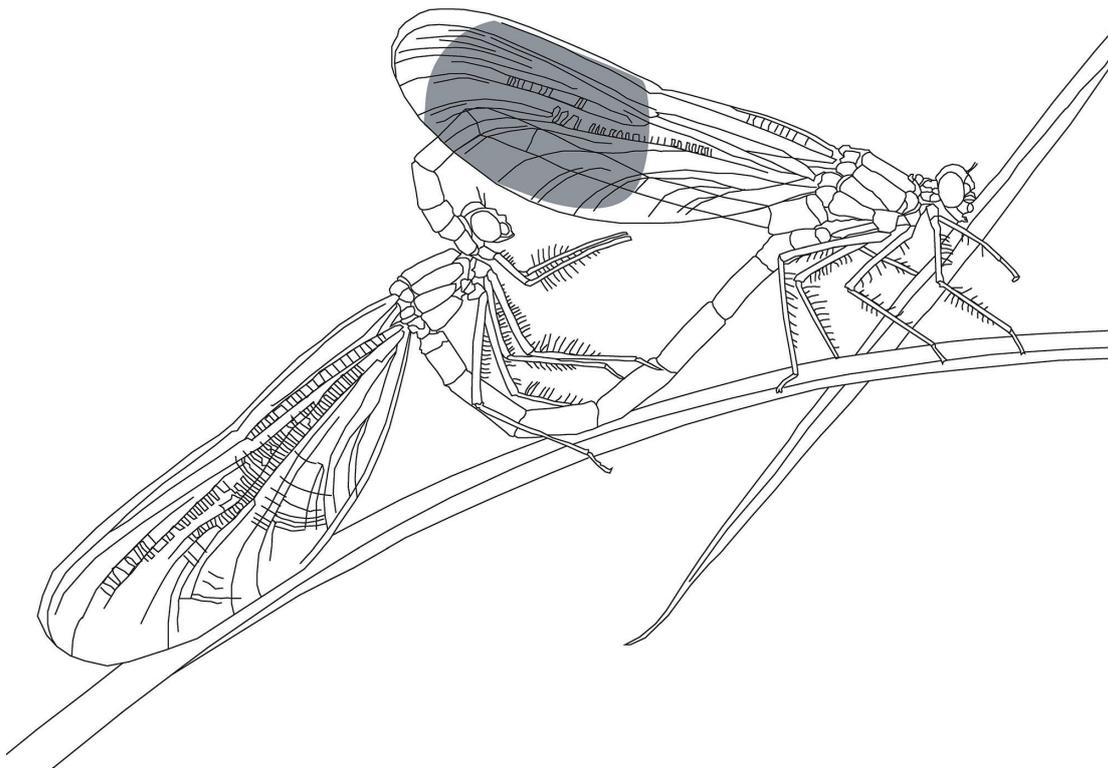


Inka Keränen

The Extent and Causes of  
Interspecific Reproductive  
Interactions in Damselflies



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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella julkisesti tarkastettavaksi yliopiston Agora-rakennuksen auditoriossa 3 kesäkuun 1. päivänä 2015 kello 12.

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UNIVERSITY OF JYVÄSKYLÄ

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# The Extent and Causes of Interspecific Reproductive Interactions in Damselflies

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Inka Keränen

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## ABSTRACT

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Diss.

As species do not live in isolation from each other, they are faced with an elementary choice when searching for a mating partner: a choice between con- and heterospecific individuals. Despite the remarkable research effort on hybridization and its avoidance, there are still some less well covered areas, e.g. what is the role of males in hybridization, what patterns are found in sympatric wild populations, what role do alternative reproductive tactics (ARTs) have on the likelihood of heterospecific matings, and what are the true costs of heterospecific interactions. In this thesis I first quantify the frequency of hybridization, backcrossing and heterospecific matings in sympatric wild populations of *Calopteryx splendens* and *C. virgo* damselflies in Finland. The possible influence of population densities, relative abundances of the species, and operational sex ratios on the frequency of heterospecific matings is also investigated. The second aim is to investigate how the intensity of territorial competition influences males' reproductive response to a heterospecific female. Finally, I dissect the importance of male ARTs on hybridization propensity and I attempt to quantify the reproductive costs that males' hybridization propensity inflicts among the tactics. The results imply a major role for *C. splendens* males in heterospecific reproductive interactions between the study species. Especially territorial males seem to be prone to hybridization, and the prevalence of hybridization is increased with a high availability of *C. virgo* females. Hybridization seems to be costly because there was high discordance between heterospecific mating frequency and observed numbers of hybrids. However, heterospecific courtship did not reduce conspecific mating success. The results also show that *C. splendens* males are able to adjust their level of heterospecific courtship according to the competitive environment as well as to the ART it follows. My thesis is a step towards understanding the causes of species reproductive interactions in wild populations.

Keywords: Alternative reproductive tactics; *Calopteryx splendens*; hybridization; microsatellite markers; reproductive isolation; territorial competition.

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- I Molecular Ecology Resources Primer Development Consortium: Knott E., Keränen I., Kuitunen K. & Wellenreuther M. *et al.* 2011. Permanent Genetic Resources added to Molecular Ecology Resources Database 1 February 2011 – 31 March 2011. *Molecular Ecology Resources* 11: 757–758.
- II Keränen I., Kahilainen A., Knott E., Kotiaho J. S. & Kuitunen K. 2013. High maternal species density mediates unidirectional heterospecific matings in *Calopteryx* damselflies. *Biological Journal of the Linnean Society* 108: 534–545.
- III Keränen I., Kahilainen A., Kotiaho J. S. & Kuitunen K. 2015. The effect of male-male competition and ornament size on mean and variance of courtship intensity towards heterospecific and conspecific females. Submitted manuscript.
- IV Keränen I., Kahilainen A., Kotiaho J. S., Heiskanen S., Holmström H., Raatikainen K. J. & Kuitunen K. 2015. Alternative reproductive tactics and the cost of hybridization in *Calopteryx splendens*. Manuscript.

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IK = Inka Keränen, KK = Katja Kuitunen, AK = Aapo Kahilainen, JSK = Janne S. Kotiaho, KEK = K. Emily Knott, HH = Hanna Holmström, SH = Siru Heiskanen, KJR = Kaisa J. Raatikainen

# 1 INTRODUCTION

## 1.1 Heterospecific reproductive interactions in nature

A first modern description of hybridization between species comes from Charles Darwin (1859). Since then, the generality and evolutionary importance of the phenomenon was questioned until the 1930s, after which scientists started to map its prevalence in nature and study hybridization experimentally (Schwenk *et al.* 2008). Nowadays, after a massive body of work on this topic, and especially due to the advent of molecular genetic tools, hybridization and heterospecific interactions have provided insights to speciation and are recognized as a powerful evolutionary force. For example, we now know that heterospecific interactions have the potential to affect species coexistence and evolution of populations (Ribeiro and Spielman 1986, Gröning and Hochkirch 2008, Schwenk *et al.* 2008, Vallin *et al.* 2012).

When populations of the same species begin to diverge, due to selection or random processes, reproductive isolation starts to accumulate between them (Coyne and Orr 2004). Therefore, reproductive interactions between individuals from these different populations become more and more costly. Hybrid offspring are often sterile or unviable, and interspecific reproductive interference can lower fitness by wasting time, energy and gametes as well as increase the risk of predation. These factors can reduce the lifespan of an individual which will have a negative effect on the lifetime reproductive success (Barton and Hewitt 1985, Verrell 1994, Svensson and Friberg 2007, Gröning and Hochkirch 2008). Thus, selection should eradicate the propensity for heterospecific reproductive interactions from the population, leading to reproductive isolation and speciation to be completed. The length of this process in nature is variable. A single mating between species can lead to origin of a new reproductively isolated species, and sometimes hybridization is observed 60 million years after divergence (Rieseberg 1997, Rothfels *et al.* 2015). These examples are provocatively extreme and come from the plant kingdom. The situation is more complex in animals and what can be said surely about the

accumulation of reproductive isolation in animals is that it increases with the time since divergence (Coyne and Orr 1989, 1997, Mallet 2005). Premating isolation (e.g. behavioral or morphological), is thought to evolve first with postmating isolation (genetic incompatibilities) following later (Mendelson 2003, Coyne and Orr 2004).

Hybridization and heterospecific courtship has been observed in many species groups (Mallet 2005, Schwenk *et al.* 2008). The reason for hybridization is usually considered to be an error in species recognition; however, sometimes it can be adaptive (Nuechterlein and Buitron 1998, Veen *et al.* 2001, Pfennig 2007). The majority of research on hybridization has focused on narrow hybrid zones and interactions between introduced and native species. This is understandable since hybrids are easier to detect from contact zones rather than large areas of sympatry and also because hybridization can threaten the existence of rare native populations (Barton and Hewitt 1985, Rhymer and Simberloff 1996). There is also a bias towards laboratory experiments. However, studies in wild populations are needed because they can give a more realistic view of the interactions and the significance of hybridization in the wild. Indeed, mate choice trials in the laboratory have been observed to sometimes overestimate the possibility and likelihood for hybridization (Hettyey and Pearman 2003, Ficetola and Bernardi 2005).

## **1.2 Male species discrimination**

### **1.2.1 Evolution and female quality**

Species discrimination is a result of the process of speciation and the development of reproductive barriers; these processes begin when some individuals start to mate selectively (Andersson 1994). This can happen due to random genetic drift in allopatry or disruptive sexual or natural selection in sympatry (Coyne and Orr 2004). When enough time has passed with selective mating, the evolved (either due to drift or selection) barriers either prevent reproduction with members of a different type or make it maladaptive (Barton and Hewitt 1985). Such selective mating is described as species discrimination. Evolution of selectivity is different between the sexes. In most species, the females' investment to reproduction and offspring is higher than that of males. Therefore, females often show higher levels of choosiness and species discrimination by females is thought to evolve before species discrimination by males (Wood and Ringo 1980, Coyne and Orr 2004). Indeed, in many cases species discrimination ability of females is found to be stronger than that of males (Andersson 1994, Noor 1996, Svensson *et al.* 2007). In most polygamous species males can increase reproductive success by increasing the number of mates, and this should deter the evolution of selectivity (Bateman 1948, Andersson 1994). Males can benefit from choice both genetically as well as directly by selecting females with attributes which will increase offspring

numbers and survival (Gwynne 1981, Schwagmeyer and Parker 1990, Puurtinen *et al.* 2009, Edward and Chapman 2011).

Mechanisms involved in recognition of suitable conspecific mates do not fundamentally differ from those used in species recognition (Andersson 1994, Pfennig 1998) and in both cases the driving evolutionary force is the fitness cost and benefit of selectivity. If the threshold for choosiness is very high, individuals can prevent mating with heterospecific females, but the likelihood for simultaneously rejecting some conspecific females increases. This can have negative effects on the lifetime reproductive success of the male (Ord *et al.* 2011). In insects there can be preference for large body size, which is often positively related to high fecundity of the female. This preference can lead to errors in species recognition when high quality conspecifics resemble heterospecific individuals (Pfennig 1998, Bonduriansky 2001). Several other factors in addition to female quality can also affect male selectivity, and variation in the propensity of males to accept heterospecific mates has been found. This variation is attributable to male parental investment, mate search costs and the competitive environment (Bonduriansky 2001, Edward and Chapman 2011).

### 1.2.2 Male parental investment

The level of investment into reproduction has been acknowledged as one of the key elements in the evolution of selectivity (Trivers 1972). There is an important distinction to be made here. Investment which directly leads to higher survival and/or number of offspring, and simultaneously lowers the individual's ability to invest in to future offspring, is expected to select for choosiness, whereas the overall investment to reproduction on its own, e.g. guarding a territory, is not (Trivers 1972, Bonduriansky 2001, Edward and Chapman 2011). It has been observed that in species that have partly or completely reversed sex roles, such as pipefish, Mormon crickets, and the threespine stickleback, male selectivity is common (Rosenqvist 1990, Gwynne 1991, Sandvik *et al.* 2000, Candolin and Salesto 2009). However, male mate choice has also been observed in species where males do not contribute to parental care (Bonduriansky 2001). For example, thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) males provide no parental care but are highly selective due to intense sperm competition associated with already mated females (Schwagmeyer and Parker 1990). When male investment to offspring is very low the cost of indiscriminate mating might also be fairly low (Trivers 1972, Parker 1983). This can lead to almost complete lack of preference for conspecific mates by males, leaving the burden of reproductive isolation on the shoulders of female discrimination ability (Noor 1996). Thus, very low parental investment from males can predispose species to hybridization, especially in systems where males are able to force matings (Tynkkynen *et al.* 2008).

### 1.2.3 Population density and relative abundance of the species

A major factor also affecting selectivity is the amount of effort required to locate a mating partner, referred as search cost (Parker 1983). If population density is very low, choosiness might not evolve (Emlen and Oring 1977, Kvarnemo and Ahnesjö 1996). This is due to the fact that potential mates are encountered rarely and rejecting any of them might lead to lower reproductive success (Bonduriansky 2001). In addition, but also independently of population density, sequential female arrival can constrain choosiness (Barry and Kokko 2010). For example, male fiddler crabs (*Uca mjoebergi*) court with equal vigour sequentially arriving con- and heterospecific females, but when females are encountered simultaneously, conspecific females are preferred (Booksmythe *et al.* 2011). If the males have no knowledge on the quality or the time lag of the consecutive female arrival, it might be a safer bet to mate with every female (Bateman and Fleming 2006). Individuals can also have diminished species discrimination ability when conspecifics are scarce and there are simultaneously many heterospecifics available (Wirtz 1999). This skewed relative abundances of two species can increase heterospecific mating attempts and hybridization (Grant and Grant 1997, Wirtz 1999, Veen *et al.* 2001, Randler 2002, Peterson *et al.* 2005, Reyer 2008). Thus, the density and relative abundance of con- and heterospecific individuals can affect the level of discrimination (Wirtz 1999, Randler 2002, Peterson *et al.* 2005).

### 1.2.4 Intrasexual competitive environment

In most sexually reproducing species, male reproductive success increases with the number of matings (Bateman 1948). All males should not prefer the same female type because this will increase competition over preferred females and decrease the likelihood of obtaining the mating (Bel-Venner *et al.* 2008). Thus, the competitive environment and male competitive ability can create variation in selectivity among males (Parker 1983, Mautz and Jennions 2011, but see Callander *et al.* 2012). Males with high competitive ability are expected to be selective since they are likely to have many mating opportunities and can benefit from selecting only the best females (Bonduriansky 2001, Edward and Chapman 2011). However, this applies only if the number of mating partners exceeds the potential mating rate of the male (Härdling and Kokko 2005). On the contrary, poor competitors have less mating opportunities and should mate with every available female or even prefer low quality females to further reduce the level of male-male competition (Bel-Venner *et al.* 2008, Venner *et al.* 2010). This could also make low quality males more susceptible to hybridization. However, an interesting pattern of prudence by small males can, at least theoretically, appear. When meeting rates with females are low, large males cannot afford to select only high quality females and due to their competitive ability, they can monopolize all females from small males. This can lead to an interesting pattern of choosiness in small males: they start targeting only high

quality females. This way, if successful, small males can gain at least some fitness benefits (Kokko and Monaghan 2001, Härdling and Kokko 2005).

An additional way of controlling one's reproductive success and taking the competitive ability into account is by alternative reproductive tactics (ARTs) (Gross 1996, Oliveira *et al.* 2008). Especially in territorial species all individuals are not able to obtain and defend a territory and some may deploy a non-territorial sneaking tactic, in which matings are obtained by deception or force (Oliveira *et al.* 2008). These tactics can be either genetically determined from birth or conditional (e.g. body size, age), meaning that individuals can opportunistically change their ART during the reproductive period (Gross 1996). Genetically determined tactics usually have equal lifetime reproductive success and their relative abundance is frequency dependent (Widemo 1998). However, when the tactics are conditional, their costs and benefits are not usually equal. Territoriality is a high cost, high benefit tactic, and for non-territorial males the cost of the tactic is lower but so is the benefit (Gross 1996, Oliveira *et al.* 2008). However, exceptions can occur. For example, in collared lizards mating success of non-territorial males can be equal to that of territorial males (York *et al.* 2014). There is evidence that ARTs can be a driver of hybridization between species, but examples of this from nature are rare (Jennings and Philipp 2002, Tynkkynen *et al.* 2009, Garner and Neff 2013).

Quantifying parental investment, mate search costs and the level of intrasexual competition can be difficult (Andersson 1994). A widely used surrogate for estimating them is the operational sex ratio (OSR), the ratio of reproductively available males and females (Emlen and Oring 1977, Andersson 1994). The sex overrepresented in the population is thought to be under stronger sexual selection and indiscriminate, whereas the sex underrepresented has an excess of potential mates and should thus be more discriminating. The strongest driver of OSR seems to be the level of parental investment: high investment leads to longer time unavailable for reproduction, skewing the OSR (Andersson 1994). This increases the mate search costs for the sex in excess, leading to stronger intrasexual competition (Trivers 1972). OSR can also be skewed due to spatial clumping of the sexes and life history traits of the species. Also, the mating system of the species affects OSR. In monogamous species with equal sex ratios OSR is close to one and sexual selection is not necessarily strong. In polygamous species, high quality individuals might have the potential to monopolize a large number of mates, making competition and sexual selection stronger (Andersson 1994). The patterns observed from nature are, however, not always this simple and there seems to be flexibility in how OSR and parental investment creates competition. For example, in katydids with high male reproductive investment (spermatophores) and female biased OSR, the pattern of competition over mates is reversed with an increase in food availability. Males are able to increase spermatophore production and mating rates which increase male-male competition and decreases female intrasexual competition (Simmons and Bailey 1990). Also, in sex-role reversed pipefish (*Syngnathus abaster*), males started to compete when OSR was experimentally manipulated to be male biased (Silva *et al.* 2010). In two-spotted gobies

(*Gobiusculus flavescens*) with mutual mate choice, male-male competition changed to female-female competition following a decrease of males in the population towards the end of the breeding season (Myhre *et al.* 2012). These examples highlight that demographics, environment and life-history of organisms can create variable patterns of selectivity that sometimes oppose the theoretical expectations.

### 1.3 Reproductive behaviour of *Calopteryx* damselflies

*Calopteryx splendens* and *C. virgo* are the only members of their genus (Order: Odonata) currently found in Finland. They have vastly overlapping geographical ranges in central- and northern Europe (Dijkstra and Lewington 2006). The species diverged five million years ago and are not considered as sister species (Dumont *et al.* 2005). There seems to be no significant niche divergence between the species when they are sympatric in Scandinavia (Wellenreuther *et al.* 2012). And at least in Finland, *C. splendens* is almost always sympatric with *C. virgo* (Tynkkynen *et al.* 2004) but *C. virgo* can occur in allopatric populations. The two species can be distinguished by the conspicuous wing coloration of the males. In *C. splendens*, the males have a metallic blue band in the middle of both fore and hind wings, whereas in *C. virgo* males, the wings are almost totally dark blue (Fig. 1). Females of these species look more alike; both have transparent wings, with *C. splendens* having a greenish and *C. virgo* a brownish tint (Dijkstra and Lewington 2006).

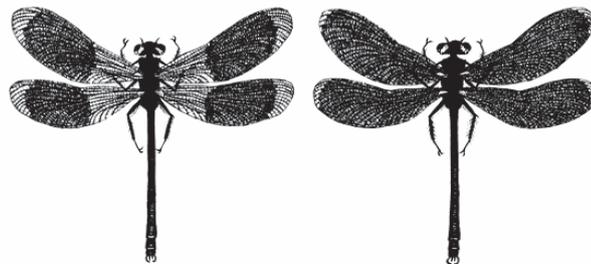


FIGURE 1 A schematic picture of male *Calopteryx splendens* (left) and *C. virgo*. Illustration by Kaisa J. Raatikainen.

The reproductive system of calopterygidae damselflies is well known (Corbet 1999, Cordoba-Aguilar and Cordero-Rivera 2005). In most species males have two different ARTs: a territorial and non-territorial tactic (the non-territorial tactic is sometimes further divided into sneaker and floater tactics) (Buchholtz 1951, Pajunen 1966, Waage 1975, 1979b, 1987, Plaistow 1997, Cordoba-Aguilar and Cordero-Rivera 2005, Koskimaki *et al.* 2009, Tynkkynen *et al.* 2009). The ART followed is determined by a male's resource holding potential and is conditional (Forsyth and Montgomerie 1987). Individual males can follow both

tactics during their reproductive life. Young males are often non-territorial because important fat reserves required for successful territorial fights have not yet been gained. Also, when males are old, they might have depleted their fat reserves and, therefore, lose their territories (Forsyth and Montgomerie 1987, Tynkkynen *et al.* 2009). Territorial males obtain mates by courting females which use the territory (floating vegetation) as an oviposition site (Buchholtz 1951, Pajunen 1966). Non-territorial males usually search for solitary females along the river (floater males) or patrol near another male's territory and steal approaching females (sneaker males) by forcing them to mate (Pajunen 1966). The main factor determining mating success in *Calopteryx* is territory ownership and territorial males have higher mating rate (Forsyth and Montgomerie 1987, Plaistow and Siva-Jothy 1996). Females are usually able to reject males (Pajunen 1966), but forced matings can occur (Cordero 1999, Rivera and Andrés 2002). Males defend their territories both intra- and interspecifically (Plaistow and Siva-Jothy 1996, Tynkkynen *et al.* 2004, 2005, 2006). The coloration of female and male wings is used as a cue in species- and mate recognition in these species (Buchholtz 1951, Tynkkynen *et al.* 2004, 2005, 2006, Svensson *et al.* 2007). The ornament size of calopterygid males is positively related to e.g. parasite resistance, fat reserves, and territory holding ability, indicating that more ornamented males may be competitively superior (Siva-Jothy 2000, Rantala *et al.* 2000, Cordoba-Aguilar and Cordero-Rivera 2005, Contreras-Garduño *et al.* 2005, 2008, Córdoba-Aguilar *et al.* 2009).

Despite the distant ancestry and wide sympatry of *C. splendens* and *C. virgo* heterospecific reproductive interactions between the species are observed in nature (Lindeboom 1996, Svensson *et al.* 2007, Tynkkynen *et al.* 2008). It seems that the species discrimination ability of females is good, but species discrimination ability of the males is quite poor (Svensson *et al.* 2007, Tynkkynen *et al.* 2008, 2009). In the majority of observed heterospecific matings, *C. splendens* has been the male partner and *C. virgo* the female (Svensson *et al.* 2007, Tynkkynen *et al.* 2008, Kuitunen *et al.* 2011). This suggests that *C. virgo* males are better at discriminating heterospecifics compared to *C. splendens* males (Svensson *et al.* 2007, Tynkkynen *et al.* 2008). There is a higher genetic incompatibility between *C. virgo* males and *C. splendens* females than in reciprocal crosses (Lindeboom 1996). Also, *C. virgo* males have higher predation risk than *C. splendens*, making visible courting display more costly to *C. virgo* than *C. splendens* (Svensson and Friberg 2007). For these reasons, selection against hybridization can be expected to be stronger in *C. virgo* males. Indeed, it has been observed that species discrimination of *C. virgo* males is better in sympatry than in allopatry (Wellenreuther *et al.* 2009).

## 1.4 Aims of the thesis

The general aim of this thesis is to provide a comprehensive image of the structure, extent and underlying reasons for hybridization between *Calopteryx splendens* and *C. virgo* damselflies in Finland.

When the decision was made to begin this thesis project there were no genetic tools available for the identification of hybrids of *Calopteryx* damselflies. Therefore, we developed reciprocally diagnostic microsatellite markers which allowed reliable identification of F1-hybrids and backcrossed individuals between *Calopteryx splendens* and *C. virgo* (I). In study II the aim is to quantitatively characterize the extent of heterospecific interactions and hybridization in the study system. The focus is to define the directionality of the matings between species as well as identify potential population level promoters for this behaviour.

Since the presence and quality of competitors as well as the male's own competitive ability can affect mating decisions by males (Fawcett and Johnstone 2003, Candolin and Salesto 2009, Mautz and Jennions 2011, Jordan *et al.* 2014), I address the effect of the immediate competitive environment on courtship behaviour of male *C. splendens* (III and IV). I test how experimentally increased territorial competition affects male courtship response towards hetero- and conspecific females (III). The territorial and non-territorial reproductive tactics of *Calopteryx* males are conditional and the mating success between them is very different (Forsyth and Montgomerie 1987, Plaistow and Siva-Jothy 1996). In study IV the main aim is to quantify the cost of heterospecific courtship to territorial and non-territorial male *C. splendens*. The second aim of study IV is to test how the different reproductive tactics affect male hetero- and conspecific courtship overall and to determine whether individual males adjust their courtship response according to the tactic they follow. The focus of this thesis is on heterospecific interactions, but I think it is important to include also the courtship response towards conspecific females (III and IV). This way the male's baseline courtship activity can be identified.

My results add knowledge about the less studied aspects of hybridization, namely: reproductive interactions between well-established sympatric species, the costs of heterospecific courtship, the role of alternative reproductive tactics in promoting heterospecific interactions and the role of males in hybridization and interspecific matings.

## 2 METHODS

### 2.1 Extent of hybridization and heterospecific interactions (I and II)

Identifying hybrids based on morphology is not a very reliable method (Mallet 2005). Therefore we developed reciprocally diagnostic microsatellite genetic markers for *Calopteryx splendens* and *C. virgo*. The loci discovered had low polymorphism, very few shared alleles and the allele sizes between species were markedly different, making these loci very useful in detecting hybrids (I).

A total of 25 sympatric populations across Southern and Central Finland were sampled to quantify the extent of hybridization between *Calopteryx splendens* and *C. virgo* (Fig. 2). Genetic data for the analysis of F1-hybrids and backcrosses was collected from 2177 individuals and population parameters (population density, species relative abundance, and operational sex ratio) were estimated during multiple visits to the sites in years 2008 and 2009 (II). Con- and heterospecific matings were also observed during these visits and when heterospecific mating was observed the male and female species were noted. Genetic analysis was performed with reciprocally diagnostic microsatellite markers (11 loci in total) to identify hybrid individuals. A maternal species analysis was also conducted for the hybrids based on universal mitochondrial 16S DNA primers (Palumbi 1996) to specify the directionality of hybridization. The effect of population parameters on con- and heterospecific mating rates was analyzed with linear regression and the deviation from the hypothesis of random mating was analyzed with Fisher's exact test (II).

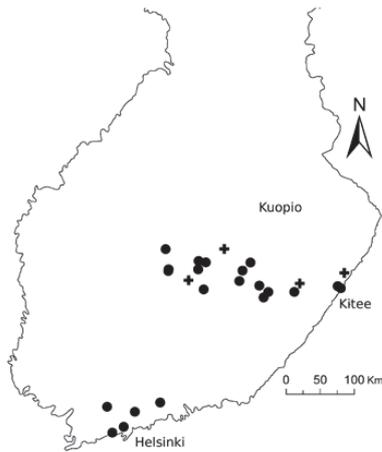


FIGURE 2 Map of study locations in Southern and Central Finland. Populations marked with crosses indicate sites where hybrids were found.

## 2.2 Male-male competition and heterospecific courtship (III)

I investigated how immediate experience of territorial competition affects the courtship response of territorial males towards hetero- and conspecific females and whether the competitive ability of the focal male modifies its response. An experiment was designed and performed in a wild population at River Niemenjoki, Central Finland in the year 2010. First, the baseline courtship response toward a conspecific female was tested by experimentally introducing a female to the territorial focal male. Second, the experience of territorial competition was created by experimentally introducing two competitor males to the focal male. Finally, immediately after the introduction of the two experimental competitors, a hetero- or conspecific female was introduced to test the focal male's courtship response. A control treatment with birch leaves (*Betula sp.*) replacing the competitor males was also conducted.

The sexual ornament (wing spot) was measured from all of the competitor males as well as the focal males to investigate how the relative competitive ability of the focal male influences its response towards the competitor males (Table 1). The effect of the focal male's ornament size on the responses towards all the females was also tested (Table 1). The main question, whether competition affects the focal males' responses towards the females, was analysed with repeated measures analysis of variance. Change in the variance of the focal males' responses towards the females was tested with Levene's test. Variance of the responses was investigated because it can reveal individual changes in behaviour due to increased competition not detectable in the mean responses.

## 2.3 Alternative reproductive tactics and hybridization propensity (IV)

*Calopteryx* males are able to switch their reproductive tactic from territorial to non-territorial and vice versa during their lifetime (Forsyth and Montgomerie 1987, Tynkkynen *et al.* 2009). The reproductive success of males following the different tactics is significantly different: territorial males obtain more matings (Plaistow and Siva-Jothy 1996). The reproductive tactic and conspecific mating success of individually marked *C. splendens* males were observed in a wild population at River Niemenjoki, Central Finland in 2011. The observed males were tested for their hybridization propensity with *C. virgo* females and conspecific courtship intensity by experimentally introducing females to them and noting the males' responses (Table 1). The aim was to determine if males with high hybridization propensity obtained less conspecific matings compared to males with low hybridization propensity. The other major objective of this study was to investigate whether males changed their hybridization propensity along with reproductive tactic. For this, when a previously tested male was observed to change its tactic, the hybridization propensity was tested again. This way we obtained data from males changing from territorial to non-territorial tactic and vice versa. We also had additional data in the analysis of tactic changers obtained from a previous study (Tynkkynen *et al.* 2009). The main question concerning the cost of hybridization propensity was analysed with zero-inflated GLM using the software R. To test the difference between overall hybridization propensity and conspecific courtship intensity between males following either territorial or non-territorial tactics, an analysis of covariance and Mann-Whitney U test was conducted. Finally, the effect of tactic change on hetero- and conspecific courtship intensity was tested with Friedman test.

TABLE 1 Response scales used in the experiments to quantify the response of the focal male: (a) level of aggression towards competitors (used only in III) and (b) willingness to mate with females (III and IV).

a) Male-Male response scale	b) Male-Female response scale
6 Physical attack	6 Tandem position or the attempt
5 Duel flight	5 Courting flight over 5 sec
4 False attack	4 Courting flight less than 5 sec
3 Warning signal	3 Interested flight toward the female
2 No response	2 No response
1 Leaves the territory	1 Warning signal

## 3 RESULTS AND DISCUSSION

### 3.1 Extent of hybridization and heterospecific interactions (II)

I identified four interspecific hybrid individuals out of the 2104 successfully analysed samples. This translates into 0.19 % prevalence of hybrid individuals in *Calopteryx* populations in Finland. One hybrid (a female) was a backcross. The sexes of the F1-hybrids were two females and a single male. A total of 272 matings were observed, 17 of which were interspecific (6 %). Interspecific reproductive interactions were unidirectional since in every case the male was *C. splendens* and the female *C. virgo*. This unidirectional pattern was further supported by the fact that all hybrid individuals contained mitochondrial DNA of *C. virgo*.

The high discrepancy between observed heterospecific matings and hybrid individuals suggests that reproductive success is low and mating between species carries high fitness costs. In a previous laboratory experiment, the egg hatching rate was not reduced in *C. splendens* male and *C. virgo* female crossings compared to conspecific crossings (Lindeboom 1996). However, since I found that adult hybrids are very rare in natural populations, developmental problems in later life stages are likely. Alternatively, it is also possible that in natural populations, females re-mate with conspecific males after heterospecific matings, and due to last male sperm precedence, hybrid offspring might not be produced (Waage 1979a, Hooper and Siva-Jothy 1996). In addition, since *C. virgo* males are able to remove sperm from the spermathecae of conspecific females, efficient sperm removal after re-mating might explain the discrepancy between heterospecific matings and hybrid individuals. In *C. splendens*, spermathecal sperm removal by males is not possible (see review in Rivera *et al.* 2004). However, there is no record of adaptive female mating behaviour in response to heterospecific matings. The single backcross found suggests that some level of genetic introgression might be possible but the probability for this is extremely low. Since females of both species are known to recognise and reject heterospecific males (Svensson *et al.* 2007), my results indicate that

heterospecific matings are more likely caused by failed species recognition and forced matings by *C. splendens* males.

The only population parameter that was significantly correlated with the rate of interspecific matings was the density of *C. virgo*. The likely explanation for this might simply be that when the density of *C. virgo* increases the likelihood for heterospecific encounters increases for the *C. splendens* males (Gröning *et al.* 2007, Svensson *et al.* 2007, Tynkkynen *et al.* 2008). The density of *C. splendens*, OSRs or the relative abundances of the two species did not correlate with interspecific matings. The OSRs were extremely male biased in both species (0.83 for *C. virgo* and 0.79 for *C. splendens*). Lack of conspecific mating partners can trigger hybridization in both sexes (Nuechterlein and Buitron 1998, Wirtz 1999, Randler 2002). In *Calopteryx*, females are essentially never lacking conspecific mating partners, adding evidence to strengthen the case that hybridization is male driven in this system. Finally, and not surprisingly, the densities of both species were positively correlated with conspecific mating rates. The males of both species fight for territories also interspecifically and *C. virgo* is dominant in these fights. *C. virgo* can cause character displacement in the sexual ornament of *C. splendens* and thus potentially have negative effects on the territorial behaviour and reproductive success of *C. splendens* (Tynkkynen *et al.* 2004, 2006, Honkavaara *et al.* 2011). However, the presence of heterospecific individuals seemed not to have negative effects on the conspecific mating rates in this study.

### 3.2 Male-male competition and heterospecific courtship (III)

The presentation of conspecific males did represent competition to the focal territorial males: the focal males responded aggressively to the presented competitor males whereas the birch leaves in the control treatment received essentially no attention at all. It was also evident that the relative competitive ability of the focal male (i.e. relative ornament size) did not affect the male's response towards the competitors. All competitor males induced similar aggressive responses from the focal males. Since territory is the major factor in determining reproductive success, and since territories are likely never regained once lost, it is not surprising that males protect this asset with high intensity against all intruders (Forsyth and Montgomerie 1987, Plaistow and Siva-Jothy 1996, Koskimaki *et al.* 2004).

The focal male's ID had a significant effect on the level of response towards the competitors. This indicates that there are some individual differences in the magnitude of territorial defence. These differences can arise e.g. from territory quality, resource-holding potential of the male and motivation and winning experience from previous encounters with competitors (Kotiaho *et al.* 1999, Hyman *et al.* 2004, Brown *et al.* 2007).

The immediate experience of territorial competition did not change the focal males' mean response towards heterospecific females. However, in the

competition treatment the variance of the responses towards heterospecifics was significantly larger compared to the control treatment. This suggests that increased territorial competition creates individual variation in hybridization propensity, and since the mean responses did not differ between competition and control treatments, some males must have increased their hybridization propensity and some decreased it. This pattern is not explained by the ornament size since it had no effect on the response towards heterospecifics. Previously, it was observed that males with large ornaments were less discriminating than males with small ornaments (Tynkkynen *et al.* 2009).

The increased variance in courtship intensity due to increased competition has also been observed in a conspecific context in three-spined sticklebacks (Candolin and Salesto 2009), but in my study, competition did not change the focal males' responses towards conspecific females or the variance in these responses. Most likely, the mating opportunities for *Calopteryx* are so rare (OSR in II) that all conspecific females always elicit a high intensity response from males. An unexpected negative covariance between the focal males' absolute ornament sizes and their responses to conspecific females was detected in my study. When the data from both competition and control treatments were pooled, results showed that males with small ornaments tended to increase their courtship response between the first and second conspecific female presented. There are other examples of males adjusting their courtship behavior according to recent social history (i.e. density and quality of females) and according to the male's own condition (Bel-Venner *et al.* 2008, Jordan and Brooks 2012). We also cannot exclude the possibility that some characteristics of the females might have affected the males' responses. However, for now, any attempts to explain this result would be story telling or speculation at best.

### **3.3 Alternative reproductive tactics and hybridization propensity (IV)**

Heterospecific courtship can have negative effects on fitness due to lost mating opportunities with conspecifics (Andrews *et al.* 1982, Singer 1990, Hochkirch *et al.* 2007). However, in this study experimentally tested hybridization propensity did not correlate with the conspecific mating rate of male *C. splendens* with either territorial or non-territorial reproductive tactics. This result indicates that heterospecific courtship does not carry major direct costs to *C. splendens* males. Territorial males had higher mating success than non-territorial males, but territorial males also responded to heterospecific females at a higher level than did non-territorials. The fact that heterospecific courtship propensity was essentially non-existent in non-territorial males could also explain why it did not correlate with conspecific mating rate. Although, in general, low quality males are expected to show indiscriminate behaviour, they can benefit from selectivity in theory (Härdling and Kokko 2005). As an example, in the yellow

dung fly (*Scathophaga stercoraria*), small non-competitive males showed higher levels of mate discrimination against non-gravid conspecific females. This was likely because differently sized males deviated in their habitat use. This way small males avoided direct male-male competition and increased their access to large females (Gress *et al.* 2014). In aquarium experiments, large successful males of three-spined sticklebacks (*Gasterosteus aculeatus*) were more willing to risk current reproductive success with sneak matings, probably due to their higher ability to replace lost offspring in comparison to small males (Candolin and Vliieger 2013).

Higher overall courtship effort has been shown to increase male mating success, and thus, territorial males might even gain from heterospecific courtship (Kotiaho 2002, Schmeller *et al.* 2005, Rosenthal 2013). It is possible that territorial males are on average in better condition in comparison to non-territorial males, and thus, can better handle the extra effort and costs of indiscriminate courtship (Koskimaki *et al.* 2009). When individual males changed tactic from non-territorial to territorial their hybridization propensity increased. However, when territorial males became non-territorial, their hybridization propensity did not decrease, but remained high. This could represent some terminal effect of compensating the low residual reproductive value of males who have lost their territory and are unlikely to regain it (Clutton-Brock 1984, Forsyth and Montgomerie 1987, Candolin 1999, Koskimaki *et al.* 2004, González-Tokman *et al.* 2013). The observed courtship patterns are not due to any intrinsic differences in reproductive activity between males following different tactics, since the conspecific courtship intensity was always high and similar between males.

## 4 CONCLUSIONS

Despite the vast amount of research directed to understanding hybridization and heterospecific matings, there is still a discordance between the theoretical expectations for the occurrence of heterospecific interactions and what is observed in nature (Schwenk *et al.* 2008, Ord *et al.* 2011). Therefore, a unified theory for heterospecific reproductive interactions is currently unavailable. It might prove to be a difficult task to accomplish, since the occurrence of heterospecific interactions varies, e. g. due to differing benefits of hybridization and heterospecific matings during the breeding season, different hybrid fitness in varying ecological conditions, learnt mating preferences, and sometimes counteracting benefits of conspecific mate choice and species discrimination (Grant and Grant 1997, Pfennig 1998, Schmeller *et al.* 2005, Heubel and Schlupp 2008, Kujtan and Dukas 2009, Rosenthal 2013, Svensson *et al.* 2014). This means that the causes for, and consequences of, species interactions are likely to depend on the environment, ecology and the social circumstances of the study system. Given these considerations, I view my results applicable only to the specified circumstances of this thesis, although similar systems might occur. However, at the same time I think that my results add an interesting example of the variety of male reproductive behaviour in nature and suggest some research ideas for the future.

Heterospecific matings between *C. splendens* and *C. virgo* seem to be mostly unidirectional, with low levels of hybridization and some possibility for introgression (II). Hybridization is most likely driven by *C. splendens* males, due to their poor species recognition, and the probability of hybridization increases with the density of *C. virgo* in sympatric populations (II). The results of this thesis and previous work on the *Calopteryx* system suggests the following reasons for the observed patterns of interspecific reproductive interactions between *C. splendens* and *C. virgo* in Finland and the poor discrimination ability of *C. splendens* males. Even though *Calopteryx* males invest highly into reproduction in general, their investment to offspring is non-existent (Corbet 1999). The investment to mating effort is not expected to select for high choosiness because it does not increase the cost of a single mating (Trivers 1972,

Bonduriansky 2001). Also the OSR is highly male biased (II), which means high competition over mates among males. Therefore, males are likely to use every mating opportunity with high intensity. In addition to the scarcity of females, males usually encounter females sequentially, which is expected to deter the evolution of selectivity (Barry and Kokko 2010). Hybridization in this system does carry fitness costs, which can be observed from the discrepancy between observed matings and hybrid individuals found in nature (II), however, some fitness returns exist because F1-hybrids are found and even backcrossing was observed (II). Heterospecific courtship might have some positive fitness effects as well: high overall courtship intensity increases the males' mating success, and in addition, I observed that hybridization propensity had no observable negative effects on conspecific mating success (IV, Kotiaho 2002, Schmeller *et al.* 2005, Rosenthal 2013).

A matter worth considering is the fact that Finnish populations of *Calopteryx* are very much on the edge of the distributional range of the species. This is especially true for *C. splendens*. My study sites are only a few hundred kilometers south from the northern edge of *C. splendens*' range; the range of *C. virgo* reaches all the way to the northern border of Finland. Populations are, in general, smaller at range edges and might be forced to live in sub-optimal habitat (Brown 1984, Gaston 2009). This might force species to live closer to each other than they would in core areas of sympatry (Wellenreuther *et al.* 2012). Therefore, heterospecific interactions might be different in the central areas of the distributional range. There is only one study reporting reproductive interactions between *C. splendens* and *C. virgo* from central Europe. In Germany, Lindeboom (1996) observed that less than one percent of observed matings in nature were between heterospecifics and all were forced matings between *C. virgo* males and *C. splendens* females; a pattern opposite to my observations. In south Sweden, most heterospecific matings are between *C. splendens* males and *C. virgo* females (2.7 %) but some reversed matings occur (0.8 %). My results showed a prevalence of 6 % heterospecific matings between *C. splendens* males and *C. virgo* females. We know from a previous study that reciprocal matings do also occur in Finland, but they have been rarely observed (Tynkkynen *et al.* 2008). This is an indication that the frequency of hybridization might be lower in more central areas of the distributional range and that the pattern might even be reversed. It is possible that due to the prevalence of open, agricultural habitats in central Europe (preferred by *C. splendens*), the two species are not as closely in sympatry as they are in Scandinavia (data, however, lacking). If this is the case, it could lead to loss of premating reproductive isolation in *C. virgo*, a pattern which has been observed from allopatric areas in north Finland (Wellenreuther *et al.* 2009).

The fact that hybridization propensity is different for males following different reproductive tactics, and moreover, that individual males change their propensity to hybridize when they change tactics from non-territorial to territorial, indicates that heterospecific courtship is not a neutral feature. *C. splendens* males might be able to adjust their accuracy of species discrimination during the male's reproductive life simultaneously with the reproductive tactic

and the intensity of territorial competition (III and IV). Studies III and IV were performed on a single population. The demographic history of the populations can affect the species discrimination ability of the individuals (Wellenreuther *et al.* 2009). Also, the population density is known to affect the territorial behavior of *Calopteryx*: in extremely high densities territoriality collapses and the majority of matings become forced (Cordero 1999). This could also decrease heterospecific mating frequencies if majority of males become non-territorial and the observed pattern (IV) of species discrimination is valid. So, it is possible that if multiple populations with different densities and relative abundances of the two species were surveyed the results might differ. An experimental manipulation of densities and relative abundances of the two species would bring vital support for my findings. It would also be interesting to quantify the males' potential mating rates and sperm limitations. This would be a step forward in understanding the upper limit of male reproductive rate and how costly occasional heterospecific matings actually are in terms of male fitness. In addition to the potential adaptive self-control of heterospecific courtship in males, another interesting avenue for future research would be to study the possible negative effects harassment of *C. splendens* might have on *C. virgo* females (Waage 1979b, McLain and Pratt 1999, Friberg *et al.* 2013).

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## YHTEENVETO (RÉSUMÉ IN FINNISH)

### Neidonkorentojen lajienvälinen risteytyminen: yleisyys ja sille altistavat tekijät

Väitöskirjassani tutkin immenkorenon (*Calopteryx splendens*) ja neidonkorenon (*C. virgo*) risteytymistä Suomessa. Tavoitteena oli selvittää risteytymisen ja lajienvälisten paritteluiden yleisyyttä sekä avata tekijöitä, jotka altistavat yksilöt tälle ilmiölle. Erityisesti tutkin immenkorentokoiraan roolia risteytymisessä ja lajienvälisissä paritteluissa. Tutkimukseni tavoitteena oli tuoda lisätietoa toistaiseksi vähemmän tutkituista risteytymiseen liittyvistä ilmiöistä. Näihin kuuluvat mm. koiraan roolin ymmärtäminen risteytymisen alullepanijana, tekijät jotka altistavat risteytymiselle samalla alueella elävillä lajeilla, vaihtoehtoisten lisääntymistaktiikoiden rooli lajienvälisissä lisääntymisvuorovaikutuksissa sekä mitkä ovat lajienvälisten paritteluiden todelliset suorat kustannukset.

Risteymien tunnistaminen ulkonäön perusteella ei ole aina luotettava menetelmä. DNA-teknologian kehityksen myötä geeneihin perustuvat risteymien tunnistusmenetelmät ovat tehokkaita ja suosittuja. Väitöskirjani ensimmäisessä osatyössä tutkimusryhmäni kehitti merkkigeenit tutkimuslajeilleni risteymien etsimistä varten. Näiden mikrosatelliittigeenien avulla pystyin luotettavasti määrittämään ensimmäisen sukupolven risteymät sekä ns. takaisinristeymät, joiden löytyminen kertoo risteymäyksilöiden kyvystä tuottaa jälkeläisiä.

Toisessa osatyössä selvitin määrällisesti risteytymisen yleisyyttä ja tutkin korreloivatko erilaiset populaatiotekijät lajienvälisten paritteluiden määrän kanssa. Kartoitin lajienvälisiä paritteluja ja keräsin DNA-näytteitä yhteensä 2177 yksilöltä 25:stä eri populaatiosta läpi Keski- ja Etelä-Suomen. Lisäksi raportoin jokaisesta populaatiosta lajien tiheydet, lukumääräsuhteen sekä lajien toiminnallisen sukupuolijakauman, eli lisääntymisvalmiiden koiraiden ja naaraiden suhteen. Kaikista tutkituista yksilöistä 0,19 % osoittautui risteymiksi. Risteymäparitteluiden vallitsevuus oli 6 % kaikista havaituista paritteluista. Lajienvälisissä paritteluissa immenkorentokoiras paritteli aina neidonkorentonaaraan kanssa. Lisäksi tutkin DNA-menetelmin löytämieni risteymien äitilajin. Tulokset vahvistivat havaitsemani yksisuuntaisen risteytymisen; kaikkien risteymien äitilaji oli neidonkorento. Mittaamistani populaatiotekijöistä ainoastaan neidonkorenon tiheys korreloi positiivisesti lajienvälisten paritteluiden kanssa. Tulokseni osoittaa, että risteytyminen on todennäköisesti haitallista yksilölle, koska havaittujen risteymäyksilöiden yleisyys oli selvästi alempi kuin havaittujen lajienvälisten paritteluiden määrä. Lajienvälisten paritteluiden määrä lisääntyy, kun lajien todennäköisyys kohdata kasvaa neidonkorenon populaatiotiheyden kasvaessa. Aiempien tutkimusten perusteella tiedämme, että sekä immen- että neidonkorenon naaraiden kyky tunnistaa oman lajinsa koiras on hyvä ja ne torjuvat toisen lajin koiraiden kosimisyriytykset. Toisaalta lajien koiraiden lajintunnistuskkyky on

heikko ja ne kykenevät ajoittain pakottamaan naaraita paritteluihin. Risteytyminen johtuukin todennäköisesti siitä, että immenkorentokoirat pakottavat neidonkorentonaaraita paritteluun.

Kolmannessa osatyössä tutkin miten kasvanut reviiirikilpailu vaikuttaa immenkorentokoiraan risteytymisalttiuteen. Reviiri on tärkein koiraan lisääntymismenestykseen vaikuttava tekijä ja siten erittäin tärkeä resurssi koiralle. Mikäli koiras kokee reviiirikilpailua, ts. uhkaa reviiirin menettämisestä, koiras voi kosiä naaraita vähemmän valikoiden. Tämä voi johtua siitä, että uusia lisääntymismahdollisuuksia ei välttämättä tule mikäli reviiiri menetetään. Tätä mahdollista koiraan kosimisaktiivisuuden muutosta testasin kokeellisesti. Koska koiraan laadulla on havaittu olevan vaikutusta sen valikoivuuteen parinvalinnassa, tutkin lisäksi koiraan laatuindikaattorin (siipitäplä) mahdollista vaikutusta kosimisaktiivisuuden muutokseen. Koeasetelmassa vein kilpailevia koiraita reviiirillisen immenkorentokoiraan reviiirille, ja välittömästi tämän jälkeen testasin koiraan halukkuutta risteytyä viemällä neidonkorentonaaraan koiraan läheisyyteen. Tuloksista selvisi, että kilpailun kokeminen ei muuttanut koiraiden keskimääräistä risteytymishalukkuutta. Kiinnostavaa oli kuitenkin se, että immenkorentokoiraiden reaktioiden vaihtelu neidonkorentonaarasta kohtaan oli merkittävästi suurempi kilpailun kokemisen jälkeen. Tämä vaihtelu ei korreloinut koiraan siipitäplän koon mukaan, joten tulokseni ei valitettavasti paljasta tämän ilmiön perimmäistä syytä. Tulos viittaa kuitenkin siihen, että kilpailu saa toiset yksilöt risteytymään halukkaammin ja toisilla risteytymisinnostus laskee. Näyttää siis siltä, että koirat voivat kontrolloida valikoivuuttaan, kun reviiirin menettämisen uhka kasvaa. Tällaisella käytöksellä voi olla positiivisia vaikutuksia koiraan elinikäisen lisääntymismenestyksen kannalta.

Neljännessä osatyössä tutkin millainen kustannus vieraan lajin kosimisesta syntyy vaihtoehtoisia lisääntymistaktiikoita noudattaville immenkorentokoiraille. Immenkorentokoirilla havaitaan luonnossa sekä reviiirillistä että ei-reviirillistä taktiikkaa. Koirat kykenevät vaihtamaan taktiikasta toiseen omasta kunnosta riippuen. Reviirillisen taktiikan energeettinen kustannus on korkea, mutta niin on myös lisääntymismenestys. Toisaalta ei-reviirilliset koirat kärsivät matalasta lisääntymismenestyksestä, mutta säästävät energiaa, koska eivät puolusta reviiiriä. Näistä syistä vieraan lajin kosiminen voi aiheuttaa erilaiset kustannukset eri taktiikkaa noudattaville koiraille. Aiemmissa, muilla lajeilla tehdyissä tutkimuksissa on selvinnyt, että vierasta lajia kosivat koirat voivat kärsiä matalammasta parittelumenestyksestä omanlajisten naaraiden keskuudessa, koska aikaa hukataan kosiessa ja paritellessa vieraslajisten naaraiden kanssa. Tässä osatyössä seurasin reviiirillisten sekä ei-reviirillisten koiraiden parittelumenestystä omanlajisten naaraiden kanssa. Seurattujen koiraiden risteytymishalukkuus testattiin kokeellisesti tuomalla elävä neidonkorentonaaras koiraan läheisyyteen. Tutkimuksen aikana taktiikkaa vaihtaneiden koiraiden risteytymishalukkuus testattiin uudestaan. Näin sain tietää muuttuuko koiraan risteytymishalukkuus sen vaihtaessa taktiikkaa. Koiraiden lisääntymismenestyksessä ei havaittu eroja risteytymishalukkaiden ja

-haluttomien välillä kummassakaan taktiikassa. Reviirilliset koiraat olivat huomattavasti innokkaampia risteytymään ja niiden parittelumenestys oli korkeampi kuin ei-reviirillisillä koirilla. Ei-reviirillisten koiraiden parempi kyky tunnistaa vieraslajinen naaras voi olla seuraus siitä, että nämä koiraat pääasiassa pakottavat naaraita paritteluun, reviirilliset puolestaan kosivat naaraita. Reviirillisen kosiessa vieraslajista naarasta se todennäköisesti torjuu kosijan, jolloin koiraan ejakulaatti säästyy. Ei-reviirillisen koiraan pakottaessa vieraslajinen naaras paritteluun se, energiakustannusten lisäksi, hukkaa myös ejakulaattia. Näin risteytymisalttiuden kokonaiskustannus voi olla pienempi reviirilliselle koiralle ja niillä ei ole tarvetta kehittää tarkkaa lajintunnistuskkyä. Kun yksilöt vaihtoivat taktiikkaa ei-reviirillisestä reviirilliseksi, niiden risteytymishalukkuus nousi. Kun muutos oli vastakkainen, koiraiden risteytymishalukkuus pysyi korkealla tasolla. Reviirinsä menettäneiden koiraiden korkea risteytymishalukkuus voi selittyä sillä, että tällaisten koiraiden tuleva lisääntymismenestys on erittäin alhainen. Tämä voi saada koiraan kosimaan kaikkia naaraita epätoivoisesti. Tulosten perusteella voimme päätellä, että vieraan lajin kosimisesta ei ole suoria kustannuksia koiralle. Lisäksi näyttää siltä, että koiraat kykenevät kontrolloimaan risteytymishalukkuuttaan vaihtaessaan lisääntymistaktiikkaa. Tämä tulos tukee myös kolmannen osatyön johtopäätöstä siitä, että koiraat voivat muuttaa risteytymishalukkuuttaan olosuhteista riippuen.

Väitöskirjani tulosten perusteella näyttää siltä, että lajienvälisiä paritteluita tapahtuu, mutta risteymien elinkelpoisuus on luultavasti alentunut. Risteytyminen on pääasiassa yksisuuntaista ja johtunee immenkorentokoiraan ja neidonkorentonaaraan välisistä pakotetuista paritteluista. Näyttää myös siltä, että korkealaatuiset reviirilliset koiraat ovat innokkaampia risteytymään kuin ei-reviirilliset koiraat. Sain myös vahvoja viitteitä koiraan kyvystä muuttaa risteytymishalukkuuttaan reviirikilpailun tason ja lisääntymistaktiikan mukaan. Kyky muuttaa innokkuutta kosia vieraan lajin yksilöä olosuhteiden mukaan voi olla koiraalle hyödyllinen ominaisuus, joka mahdollisesti lisää elinikäistä lisääntymismenestystä.

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IV

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