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RICE UNIVERSITY

POLYANDRY AS A HEDGE AGAINST GENETIC INCOMPATIBILITY

by

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ABSTRACT

Polyandry as a Hedge Against Genetic Incompatibility

by

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Why do females across a wide range of taxa mate with more than one male? Here, I present the hypothesis that females engage in polyandry as a hedge against genetic incompatibility. I review evidence from the literature showing that the genomes of species are dynamic entities, constantly evolving as a consequence of genetic conflicts within and between the nucleus and the cytoplasm. Cellular endosymbionts, segregation distorter alleles, transposable elements and genomically-imprinted genes can all threaten female fitness by modifying maternal and paternal haplotypes in ways that render them incompatible within the developing embryo. I discuss the potential for polyandrous females to utilize postcopulatory mechanisms such as sperm competition, female choice of sperm, and reallocation of maternal resources from defective to viable embryos in order to minimize the risk and/or cost of fertilization by genetically-incompatible sperm. In a sperm precedence experiment carried out on the pseudoscorpion, Cordylochernes scorpioides, single-locus minisatellite DNA fingerprinting demonstrated strong last-male sperm precedence when females were mated to two males which broke down
completely when females were mated to three males. This result indicates that the opportunity for postcopulatory sexual selection may be much greater in nature than is evident from standard, laboratory, two-male mating experiments. Polyandry in this pseudoscorpion is shown to be a deliberate strategy which increases reproductive success. In laboratory experiments, females restricted to mating with a single male experienced a higher rate of embryo failure and produced significantly fewer offspring than either females mated to more than one male in the laboratory or females naturally inseminated in the field. Previously proposed hypotheses such as forced copulation, insufficient sperm from a single mating, male nutrient donations, offspring genetic diversity and inherent male genetic quality cannot explain this higher reproductive success of polyandrous females. Observations of meiotic drive, highly-skewed sex ratios and paternal effects on sex ratio in this pseudoscorpion are consistent with the hypothesis that, by accumulating sperm from several males, *C. scorpioides* females reduce the number of embryos which fail as a consequence of genetic incompatibility between maternal and paternal genomes.
ACKNOWLEDGMENTS

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PREFACE

At its most fundamental level, gender is defined by the relative size of the sexual cells. In all sexually-reproducing species, females produce a limited number of large, costly eggs while males produce many small, cheap sperm. In his landmark paper, Trivers (1972) argued that this sexual disparity in gamete investment (anisogamy) predisposes males and females to adopt very different optimal mating strategies. Since any single female manufactures relatively few eggs, a male can best enhance his reproductive success by mating with as many females as possible. By contrast, a female should safeguard her greater physiological investment in gametes by being extremely selective in her choice of mate. Having chosen the most attractive or highest quality male available, she then derives no reproductive benefit from copulating with additional males. Her mate can provide more than enough sperm to fertilize all her eggs (Williams 1966; Parker 1992).

This model of female monogamy and male polygamy has provided the paradigm for two decades of research in the field of sexual selection. However, the recent development of powerful molecular tools for assigning paternity in natural populations (e.g., Jeffreys et al. 1985; Bruford et al. 1992) is revealing that multiple paternity, and hence polyandry (mating with more than one male), is a pervasive phenomenon in animals as diverse as snakes (Schwartz et al. 1989), birds (Dunn & Lifjeld 1994) and pseudoscorpions (Zeh & Zeh 1994b). In one of the most extreme cases of multiple paternity so far detected, 44% of broods in the aquatic warbler consisted of offspring sired by three or four males.
(Schulze-Hagen et al. 1993). Even in birds thought to be exclusively monogamous, DNA fingerprinting has revealed that females frequently mate with more than one male (see Wetton et al. 1992). Similarly, in mating systems apparently dominated by male competition, molecular studies are revealing that females, in fact, pursue their own covert agenda for acquiring fathers for their offspring. In grey seals, for example, DNA fingerprinting has shown the reproductive success of dominant, harem-defending males to be significantly lower than expected from field observations of copulation, suggesting that females often mate with subordinate males outside the breeding colony (Amos et al. 1993). This growing evidence of widespread polyandry calls for a critical reassessment of the selective forces which act to shape female mating tactics.

I suggest that a better understanding of the causes of polyandry may be gained by considering the implications of intragenomic conflict for female reproductive success. Discoveries in molecular biology are revealing that the genomes of species are dynamic entities, constantly evolving as a consequence of genetic conflicts within and between the nucleus and the cytoplasm. Cellular endosymbionts, segregation distorter alleles, transposable elements and genomically-imprinted genes can all undermine female fitness by rendering certain combinations of maternal and paternal haplotypes incompatible within the developing embryo. In the following chapters, I present the hypothesis that, by accepting sperm from several males, promiscuous females are able to utilize postcopulatory mechanisms such as sperm competition, female choice of sperm, and reallocation of maternal resources
from defective to viable embryos in order to minimize the risk and/or cost of fertilization by genetically-incompatible sperm.

In chapter 1, I develop the genetic incompatibility hypothesis by reviewing circumstances in which intragenomic conflict can generate intrapopulation incompatibility and thereby threaten female reproductive success. This is followed by discussion of the ways in which polyandry may act to minimize this threat. Chapters 2 and 3 focus on my empirical research on female mating behavior in the harlequin beetle-riding pseudoscorpion, Cordylochernes scorpoides. The recent development of single-locus minisatellite DNA probes for this arachnid (Zeh et al. 1994), in combination with a reproductive biology highly amenable to experimentation and involving the production of large numbers of offspring (Zeh & Zeh 1994a), makes this species a model system for investigating the causes and consequences of polyandry. In Chapter 2, I present the results of an experiment which demonstrated that when females mate with several males, male mating order does not determine which sperm fertilize eggs. This absence of mating order constraints is an important prerequisite for the genetic incompatibility hypothesis since it suggests that the opportunity for postcopulatory sexual selection is much greater in nature than is evident from previous laboratory sperm precedence experiments involving only two males. In chapter 3, I show that polyandry in C. scorpoides is a deliberate strategy that increases female reproductive success. By eliminating inherent male quality and other previously proposed hypotheses, this research points to the threat of genetic incompatibility as a force driving the evolution of multiple
mating by females.

Finally, chapter 4, which considers the genetic basis of clonal competitive ability in the sea anemone, *Anthopleura elegantissima*, is included here for two reasons. First, it provides an example of how the invertebrate immune system can discriminate between self and non-self. Without the capacity for such discriminatory ability, there would clearly be little scope for postcopulatory sexual selection through female choice of sperm. Second, it illustrates the importance of incorporating an understanding of underlying genetic mechanisms into any interpretation of behavioral, ecological and evolutionary patterns.
CHAPTER 1

INTRAGENOMIC CONFLICT AND GENETIC INCOMPATIBILITY:
A NEW HYPOTHESIS FOR POLYANDRY

1. INTRODUCTION

Fundamental to sexual selection theory has been the premise that sexual disparity in gamete investment (anisogamy) generates selection for divergent mating tactics in males and females (Trivers 1972). Males can generally maximize their reproductive success by mating with many females. By contrast, females best safeguard their investment in eggs by choosing the most attractive or highest quality male (Fisher 1930; Williams 1966; Parker 1992). The adequacy of this view of female mating tactics is coming under increasing scrutiny, however, as a consequence of growing molecular evidence that multiple paternity, and hence polyandry, is widespread in nature (Smith 1984b; Ginsberg & Huck 1989; Birkhead & Møller 1992; Dunn & Lifjeld 1994). Although polyandry in many species can be explained within the framework of promiscuous males and essentially choosy females (see section on alternative hypotheses), in other cases, females appear to mate multiply as a deliberate strategy for acquiring sperm from several different males (Amos et al. 1993; Schulze-Hagen et al. 1993; Ligon & Zwartjes 1995; Zeh chap. 3). As the focus
of behavioral ecology shifts increasingly to consideration of reproduction from
the female point of view (Gowaty 1994) and as more data become available,
determining the extent and causes of polyandry is likely to have a major impact
on future developments in sexual selection theory.

Various hypotheses have been put forward to explain why females mate
with several males, and these can be broadly categorized according to whether
they involve material or genetic benefits (see below). The diversity of these
hypotheses illustrates the complexity of forces thought to influence female
mating tactics, and many of the proposed factors undoubtedly do contribute, in
particular situations, to selection for multiple mating by females. Nonetheless,
nearly all previously proposed hypotheses are based on the tacit assumption
that the nuclear and cytoplasmic genomes of any female in the population can
be combined with the nuclear genome of any male to produce a viable zygote
which can complete embryonic development and attain sexual maturity. This
assumption seems increasingly unrealistic. Discoveries in molecular biology
are providing growing support for the view that intragenomic conflict is a
fundamental driving force in evolution (Werren et al. 1988; Hurst 1992;
Saumitou-Laprade et al. 1994; Domínguez 1995). It is becoming evident that
the genomes of species are not at static equilibrium. They are dynamic entities,
constantly evolving at least in part as a result of genetic conflicts within and
between the nucleus and the cytoplasm. As a consequence, any female setting
out to mate and reproduce is faced with an array of males likely to vary in the
extent to which they are reproductively compatible with her own genotype.
Incompatibility between paternal and maternal genotypes has been implicated in spontaneous embryo abortion in flowering plants (Wiens et al. 1987; Zuberi & Lewis 1988), and could be a factor contributing to the relatively high rates of early embryo failure detected in a number of animal species. For example, in humans, it is estimated that approximately 10-15% of clinically evident pregnancies undergo spontaneous abortion (Fox & Buckley 1992), and that, overall, only 42% of fertilized eggs survive to the twelfth day of pregnancy (Hertig & Rock 1959; Hertig et al. 1959). Similarly high estimates of early fetal loss, ranging from 10% to 60%, have been obtained for other mammal species (reviewed in Baker & Bellis 1995).

If genetic incompatibility is a significant factor influencing female reproductive success, then, as Parker (1992) has pointed out, "... to expect selection to produce mate choices which take account of the combined result of a female's genotype and that of her suitor is indeed to have faith." Here, I propose that multiple mating may provide females with at least a partial solution to this predicament. By mating with several males, females may be able to exploit postcopulatory mechanisms, such as sperm competition, female choice of sperm, and reallocation of maternal resources from defective to viable embryos, in order to minimize the risk and/or cost of fertilization by genetically-incompatible sperm. After briefly summarizing alternative hypotheses, I develop the genetic incompatibility hypothesis by reviewing circumstances in which intragenomic conflict can threaten female reproductive success, followed by discussion of the ways in which polyandry may act to minimize this threat.
2. ALTERNATIVE HYPOTHESES

Several hypotheses have been proposed to explain why females mate with more than one male, despite potentially high costs (Chapman et al. 1995; Keller 1995). Females may simply be unable to prevent forced copulation (Thornhill 1980). They might not obtain sufficient sperm from a single mating (Ridley 1988). In socially monogamous species, females can sometimes engage in extra-pair copulations to compensate for a mate of low fertility (Wetton & Parkin 1991) or poor quality (Westneat et al. 1990; Kempanaers et al. 1992). Females may mate multiply in order to obtain material benefits such as nutrients from spermatophores or seminal fluid (Zeh & Smith 1985; Simmons 1992; LaMunyon & Eisner 1993; Bissoondath & Wiklund 1995) or additional paternal care (Davies 1992). Mating with several males could confer some form of genetic advantage. For example, it has been suggested that the increased offspring diversity resulting from multiple paternity enhances female fitness (Loman et al. 1988; Ridley 1993; Keller and Reeve 1994). Alternatively, polyandry, in combination with a reproductive tract hostile to sperm, may enable sperm competition or cryptic female choice to increase the probability that eggs are fertilized by high-quality sperm or sperm from high-quality males (Madsen et al. 1992; Birkhead et al. 1993). Genetic benefits may derive from Fisherian sexual selection in which the sons of multiply-mated females produce competitively superior sperm or ejaculates (the sexually-selected sperm hypothesis: Keller and Reeve 1995). Finally, polyandry may diminish the cost of
inbreeding in situations where females either cannot recognize close relatives or are unable to avoid mating with them (Stockley et al. 1993). These previously proposed genetic benefit hypotheses make several predictions which differ from those which follow from the genetic incompatibility hypothesis (Table 1.1). Most significantly, with the exception of the inbreeding avoidance hypothesis, they do not predict any increase in offspring number resulting from multiple matings by females.

3. INTRAGENOMIC CONFLICT AND GENETIC INCOMPATIBILITY

Expanding on a theory originally proposed by Cosmides and Tooby (1981) and Werren et al. (1988), Hurst (1992) and Hurst et al. (1992) have argued that intragenomic conflict could act as a driving force in the evolution of sex, meiotic mechanisms, recombination, genome size, genomic imprinting, sex ratio, sex allocation systems, sex determining systems and postzygotic isolation. Here, I extend the theory further by suggesting that intragenomic conflict may also be an important force driving the evolution of multiple mating by females. In this section, I discuss the capacity of cellular endosymbionts, transposable elements, meiotic drive alleles, and imbalance between genomically-imprinted genes to interfere with embryonic development and/or endanger maternal health. As I emphasize below, the extent to which these various agents of intragenomic conflict actually have an impact on the reproductive success of a
Table 1.1. Comparison of the predictions of genetic benefit models for the evolution of polyandry. The inbreeding avoidance hypothesis makes the same predictions as the genetic incompatibility hypothesis but the mechanisms producing incompatibility differ. Note that RS = reproductive success.

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<tr>
<th>CONTEXT</th>
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<tr>
<td></td>
<td>Inherent Male or Sperm Genetic Quality</td>
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<tr>
<td>Variance In Male or Sperm Fitness</td>
<td>High</td>
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<tr>
<td>Variance in Male Phenotype</td>
<td>Polyandry most important when low</td>
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<td>Embryo Failure Rate</td>
<td>Low</td>
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<tr>
<td>Inherent Male or Sperm Genetic Quality</td>
<td>Sexualy-Selected Sperm Hypothesis</td>
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<tr>
<td>Correlation Between Rs of Females Mated to Same Male</td>
<td>High No specific prediction</td>
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<tr>
<td>Reproductive or Developmental Mode</td>
<td>No specific prediction</td>
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<tr>
<td></td>
<td>Inherent Male or Sperm Genetic Quality</td>
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<tr>
<td>Reproductive Outcome</td>
<td>Higher quality offspring</td>
</tr>
<tr>
<td>Form of Selection in Female Reproductive Tract</td>
<td>Positive selection for best male type</td>
</tr>
<tr>
<td>Extent Of Resulting Multiple Paternity</td>
<td>Low</td>
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female depends critically on both the genetic characteristics of her oocyte and those of the penetrating sperm.

The genetic incompatibility hypothesis differs from other genetic benefit models of multiple mating by females in two significant ways. First, the fitness consequences of intragenomic conflict in all cases depend on an interaction between maternal and paternal haplotypes, or in the language of population genetics, fitness effects are non-additive. This undermines the notion of female choice based exclusively on inherent male genetic quality. Second, endless cycles of reciprocal evolutionary change brought about by conflicts of interests
between competing genetic elements (Hurst 1992) are likely to ensure the continual input of genetic incompatibility as a threat to female reproductive success.

(a) Cellular endosymbionts

Genetic elements located in the cytoplasm are typically transmitted only through females. Males therefore represent a dead end for cellular endosymbionts such as protozoans, bacteria and viruses which can increase their transmission rate by biasing sex ratio in favor of females (Cosmides and Tooby 1981). Female bias, in turn, generates selection on biparentally-inherited nuclear genes to restore the sex ratio to 1:1, setting in motion an evolutionary arms race fueled by nucleo-cytoplasmic conflict over optimal sex ratio (Hurst 1991). Cellular endosymbionts can distort sex ratio by either killing or feminizing male embryos (Hurst 1993). Although sabotaging male offspring appears to be an inefficient mechanism for distorting sex ratio, cytoplasmic male killers are known or suspected to be agents of sex ratio bias in a wide variety of arthropods, including amphipods, isopods, scale insects, fruit flies, wasps, mites, butterflies, beetles and bugs (reviewed in Ebbert 1993; Hurst & Majerus 1993; Hurst 1993). The frequency of such male killers varies between populations as well as between species, with, for example, approximately 4% of individuals in natural populations of Nasonia vitripennis being affected (Skinner
1985), compared to 13% of females in *Drosophila prosaltans* populations in Brazil (Cavalcanti et al. 1958), and, in the Egyptian cotton leafworm, *Spodoptera littoralis*, as many as 20-30% of females in some areas (Brimacombe 1980). Clearly, females infected with male killers face a significant reduction in their reproductive success since, even if they can reallocate resources to daughters, they are unlikely to be able to fully compensate for the death of their sons. By comparison, since feminizing factors do not reduce offspring number, their impact on female fitness is more insidious. Known to occur in isopod and amphipod crustaceans (Juchault & Legrand 1989), feminizing, cellular endosymbionts could reduce female fitness if the overall sex ratio of the host population becomes sufficiently female-biased. With males at a premium, infected females would have lower fitness than females still capable of producing sons (Charnov 1982).

Certain cellular endosymbionts in the bacterial genus *Wolbachia* (Werren et al. 1995) spread through populations without distorting sex ratio by creating a form of postzygotic reproductive incompatibility known as cytoplasmic incompatibility (Fine 1978). In this case, the endosymbiont increases in frequency by putting uninfected females at a reproductive disadvantage. Whereas females harboring the endosymbiont are compatible with both infected and uninfected males, uninfected females produce inviable embryos if their ova are fertilized by sperm from infected males (Barr 1980). *Wolbachia* occurs in at least seven orders of insects and the phylogenetic distribution of *Wolbachia* types provides extensive evidence of horizontal transmission.
between host taxa (Werren et al. 1995). The ability of these cellular
endosymbionts to generate cytoplasmic incompatibility in new hosts has been
demonstrated by recent microinjection experiments (Braig et al. 1994; Giordano
et al. 1995).

(b) Transposable elements

Transposable elements are mobile DNA parasites which increase their copy
number by inserting into new sites within the nuclear genome of their host (Berg
1989; Robertson & Lampe 1995). There is growing evidence that DNA-
mediated transposable elements such as P and mariner may persist primarily
through repeated episodes of horizontal transfer between species (Robertson &
Lampe 1995). During the initial phase of establishment within a new host,
selection is likely to favor those elements which scatter copies of themselves
throughout the genome, thereby reducing the risk of elimination by deletion
events (Hurst et al. 1992). For the host, however, high levels of transposition
increase the probability of functional gene disruption and are likely to be
deleterious (Charlesworth & Langley 1991). As a consequence, selection
operating at the genetic and phenotypic levels can result in intranuclear conflict
(Hurst et al. 1992). Perhaps the most direct evidence that such conflict actually
occurs comes from crosses between transposon-free Drosophila strains and
strains possessing such mobile elements as P, I, hobo and mariner (e.g.,
Kidwell et al. 1977; Blackman et al. 1987; Maruyama & Hartl 1991; Petrov et al. 1995). Patterns of reproductive compatibility in reciprocal crosses are similar to those observed in cases of *Wolbachia*-mediated cytoplasmic incompatibility. For example, females possessing *P* elements are compatible with both *P*-carrying males and so-called *M*-strain males which lack the elements. By contrast, matings between *M* females and *P* males produce progeny exhibiting high rates of mutation, chromosomal rearrangement and sterility due to abnormal gonadal development (Rio 1991). This hybrid dysgenesis involves both chromosomal and cytoplasmic components (Lozovskaya et al. 1995). Whether or not transposable elements reduce the fitness of a particular female thus depends on a complex interaction between both her genotype and cytotype and the nuclear genes of her mate.

*(c) Meiotic drive*

Conflict between different alleles of a nuclear gene can result in meiotic drive, a process occurring during gametogenesis in which one type of allele, the driver, sabotages gametes carrying alternative alleles. Meiotic drive has been shown to occur in a wide range of organisms from slime molds (Clark & Landolt 1993) to mice (Silver 1993). In the two most thoroughly studied cases, the *SD* locus in *D. melanogaster* (McLean et al. 1994), and the t-complex region in *Mus musculus* (Lenington et al. 1994), meiotic drive is associated with genotype-
dependent negative fitness consequences, with homozygosity for the drive allele leading to embryo death or, in adults, sterility or reduced fertility. As Fig. 1.1 illustrates, heterozygous females mating with heterozygous males may suffer up to 50% reduction in their reproductive success because, although segregation in females is normal, nearly all the viable sperm (90-99%) produced by heterozygous males carry the drive allele (Lyttle 1991). By contrast, females not themselves carrying the drive allele experience no direct fitness cost to mating with heterozygous males. The general importance of meiotic drive as a threat to female reproductive success remains to be determined. It certainly represents a significant risk in mice where t-haplotypes occur with a frequency of 10-20% in natural populations (Lyttle 1991). Unfortunately, because it is difficult to detect, there is no accurate estimate of the general importance of meiotic drive. The fact that segregation distortion systems have been found in many of the species for which large pedigrees and genetic markers are available suggests that this form of intragenomic conflict may be much more common than is currently appreciated (Hurst & Pomiankowski 1991).

(d) Genomic imprinting

Haig & Westoby (1989) have proposed that the ‘placental habit’ (Harper et al. 1970), common to both angiosperm and mammalian development, is
Fig. 1.1. Schematic diagram of the effects of mating between a male and female heterozygous at a meiotic drive locus. Whereas the majority of the male's sperm carries the distserter allele, meiotic drive does not occur in the female and the distserter and wild-type alleles are equally represented in her eggs. Mating between heterozygotes significantly reduces the female’s reproductive success since homozygous embryos die or, alternatively, in the case of males, develop into sterile individuals. By contrast, this effect of mating with a drive allele-carrying male is not experienced by females homozygous for wild-type alleles who produce embryos which are all heterozygous and therefore normal.
responsible for the evolution of genomic imprinting in both taxa. This term describes a process in which certain genes exhibit parental-origin-dependent expression (reviewed in Stewart 1993 and Matzke & Matzke 1993) which is correlated with differential methylation of DNA during gametogenesis in males and females (Li et al. 1993). In embryonic development, maternally-inherited alleles at imprinted loci may exhibit patterns of expression which are radically different from those exhibited by their paternally-inherited counterparts. It has been hypothesized that imprinting results from an intragenomic "tug-of-war" in which paternally-inherited genes promote nutrient transfer from the mother to the embryo, while maternally-inherited genes act to suppress this activity (Haig & Westoby 1989; Moore & Haig 1991). As illustrated in Fig. 1.2, in the embryonic and extraembryonic tissues of mice, for example, insulin-like growth factor II (IGF-II), which stimulates nutrient transfer from the mother, is transcribed only from the paternal copy of the Igf2 gene (DeChiara et al. 1991). This pattern is reversed at the Igf2 mannose-6-phosphate receptor locus (Igf2/MPR) where the paternal allele is inactive and transcription of the maternal allele results in degradation of IGF-II (Barlow et al. 1991). A similar process occurs in humans (Schneid et al. 1993; Zhang et al. 1993). In plants, genetic evidence from maize suggests that imprinting occurs at many developmentally critical endosperm loci (Haig & Westoby 1991). Development thus involves a delicate balance between the opposing effects of maternal and paternal genes. Point mutations, deletions and chromosome rearrangements which disrupt normal patterns of imprinting are known to have profound phenotypic effects, ranging from embryonic death
Genomic Imprinting

Fig. 1.2. Schematic diagram of patterns of genomic imprinting of the insulin-like growth factor 2 gene (Igf2) and the mannose-6-phosphate receptor locus (MPR). In the embryo, only the paternally-inherited copy of the Igf2 gene is expressed and promotes the transfer of nutrients from the mother. The pattern of imprinting is reversed at the MPR locus where expression of the maternally-inherited allele results in degradation of IGF-II and thus reduction in the amount of nutrients transferred. The combination of a weakly-expressed paternal Igf2 allele with a strongly-expressed maternal MPR allele is likely to severely impede embryonic development.

Clearly, such conditions are pathologies unlikely to affect more than a small percentage of individuals in any population but does the process of genomic imprinting itself carry an inherent threat to female reproductive success? I suggest that it may. It is evident from recent studies that the process of nutrient transfer from mother to embryo is a complex one, involving interplay between numerous imprinted genes, as well as chromosomal domain events (Nicholls 1994). Assuming the evolutionary tug-of-war hypothesis to be correct, the various components of this interplay are constantly evolving as a consequence of conflict between paternal and maternal genes. If resource promotion activity by the paternal genome and suppression by the maternal genome are viewed as quantitative traits controlled by several loci (Fig. 1.2), it seems inevitable that maternal and paternal genomes will vary in the extent to which they are genetically compatible. For example, a maternal multilocus genotype of high suppression activity will produce normal offspring only when paired with paternal genotypes of equally vigorous resource transfer activity (Fig. 1.2). Matings between males and females on the opposite ends of their respective distributions of genic expression are likely to result either in overdemanding progeny that pose a threat to maternal health (see Haig 1993) or in embryos unable to complete normal development.

The consequences of imbalances between maternal and paternal imprinted
genes are manifested in their most extreme form in human complete hydatidiform moles (CHMs) which in most cases develop from conceptuses possessing a diploid set of chromosomes which are all paternal in origin (Kovacs et al. 1991). Characterized by excessive proliferation of placental tissue and a complete absence of any fetus, CHMs have been hypothesized to result from inactivation or loss of maternal genetic material in the fertilized ovum and duplication of the fertilizing sperm’s genome (Kajii & Ohama 1977). Partial hydatidiform moles (PHMs) which generally exhibit less extreme morphological imbalances between fetal and placental growth, possess both maternal and paternal genomes but in abnormal ratios (Szulman & Surti 1978). A recent estimate indicates that hydatidiform moles may be much more common than previously supposed and may affect approximately 1% of all clinically-evident pregnancies in some populations (Jeffers et al. 1993).

4. POSTCOPULATORY DEFENSES AGAINST INCOMPATIBILITY

It is clear that cellular endosymbionts, transposable elements, meiotic drive and genomically-imprinted genes all have the potential to severely impact on female fitness. It is also evident that the extent to which this threat is realized for any given female depends on how her nuclear and cytoplasmic genotypes interact with the nuclear genes of her mate. The importance of ‘genic balance’ has long been recognized in plants (Haig & Westoby 1991). However, with few
exceptions (e.g., Smith 1984a; Stockley et al. 1993), evolutionary biologists have failed to appreciate the significance of intrapopulation reproductive incompatibility and its implications for female mating strategies in animals, perhaps because it has not been obvious how females could go about evading such a threat. Precopulatory mate choice based on male phenotype appears to provide little scope for females to match male genotype against their own in order to avoid having their eggs fertilized by genetically-incompatible sperm (Parker 1992; but see Drickamer & Lenington (1987) for an exception). Similarly, until recently, apparently strong mating order effects on sperm utilization (Birkhead & Hunter 1990) provided little scope for mechanisms operating at the postcopulatory stage. However, this view is now being undermined by increasing molecular evidence that multiple paternity is widespread in nature, with data currently available on many species of birds (reviewed in Birkhead & Möller 1995), several mammals (Inoue et al. 1990; Tegelström et al. 1991; Amos et al. 1993; Murie 1995; Schenk & Kovacs 1995), as well as some snakes (e.g., Still et al. 1986; Schwartz et al. 1989), turtles (Galbraith 1993), isopods (Heath et al. 1990), insects (Gromko et al. 1984; Moritz et al. 1995; Oldroyd et al. 1995), spiders (Martyniuk & Jaenike 1982; Oxford 1993), and pseudoscorpions (Zeh & Zeh 1994).

In addition, recent experiments on pseudoscorpions (Zeh & Zeh 1994) and mites (J. Radwan, personal communication) have shown that last-male sperm precedence can be an artefact of two-male, laboratory mating experiments. In other species, the extent of mating effects on sperm utilization is know to vary,
depending on the mating context (Siva-Jothy & Tsubaki 1989; Radwan 1991; Bauer 1994; Otronen 1994). The relaxation of mating order constraints on sperm utilization when females mate with several males suggests that the opportunity for postcopulatory sexual selection may be much greater than previously supposed. In this section, I present examples to illustrate how polyandry and accumulation of sperm from several males could enable females to exploit sperm competition, female choice of sperm and re-allocation of maternal resources in order to safeguard their reproductive investment against the threats posed by intragenomic conflict. The majority of these examples involve meiotic drive simply because this form of intragenomic conflict has been extensively characterized in both the mouse and D. melanogaster.

(a) Sperm competition

It has been argued that in the adder, Vipera berus, sperm competition increases the probability that the eggs of polyandrous females will be fertilized by sperm of high genetic quality (Madsen et al. 1992). Sperm competition also provides females with a mechanism for reducing the probability of fertilization by genetically-incompatible sperm. As Wu (1983) was the first to point out for Drosophila, in meiotic drive systems, heterozygous males are likely to be at a disadvantage in sperm competition since they produce up to 50% fewer viable sperm per ejaculate than males not carrying the drive allele. Haig & Bergstrom
(1995) have argued that, if females mate with several males, this handicap could act as a check on the spread of selfish meiotic drive alleles, with genes that promote multiple mating by females being selected to reduce the advantage of the transmission distorting allele. From the standpoint of meiotic drive's negative impact on female fitness, sperm competition is most advantageous for females who are themselves heterozygous at the drive locus. In addition to paying the indirect cost associated with the lower competitive ability of heterozygous sons, heterozygous females face the more immediate risk of producing offspring which are homozygous at the drive locus and consequently inviable or infertile. In fact, it is this direct cost to heterozygous females that is likely to drive the evolution of multiple mating. In a population of initially monogamous females, sperm competition would not occur, and selection on genes that promote polyandry would operate only in heterozygous females.

(b) Female choice of sperm

Recent experiments involving crosses between closely-related and partially reproductively-compatible species of grasshoppers (Hewitt et al. 1989), crickets (Howard & Gregory 1993) and beetles (Wade et al. 1994) suggest that postcopulatory sexual selection may play an important role in preventing the production of defective offspring. Although at least some viable, hybrid offspring
were produced from heterospecific crosses, when females mated with both a conspecific and a heterospecific male, any mating order effects were overridden and eggs were fertilized by conspecific sperm (the most genetically-compatible). This could be the result of sperm competition in which conspecific sperm are better adapted to negotiate the female reproductive tract (Eberhard 1996). Alternatively, females may be able to recognize differences between sperm genotypes and either actively choose sperm to be used in fertilization or bias against certain genotypes through inhibition or preferential sperm loss (Zimmering et al. 1970). Although few studies have been directly aimed at investigating the mechanisms of non-random sperm utilization, there is evidence for compatibility-based discrimination against sperm genotypes in the female reproductive tracts of *Drosophila*, flour beetles, mice, rabbits and humans (see Table 1.2).

How might such female choice of sperm genotype occur? It is now known that, in mammals, a number of cell-surface proteins of spermatozoa are synthesized through haploid gene expression during spermiogenesis (e.g., Klemm et al. 1989; Erickson 1991; Penttilä et al. 1995; Choudhary et al. 1995). These macromolecules can stimulate the production of autoantibodies in males, and are normally sequestered from the immune system by the blood-testis barrier (Bellvé et al. 1990). After transfer to the female, sperm are perceived as antigens and must run the gauntlet of a female reproductive tract populated by large numbers of antisperm leukocytes and antibodies (for a review of female barriers to sperm, see Birkhead et al. (1993)). As a consequence, of the 40 to
Table 1.2. Studies providing evidence for female choice of genetically-compatible sperm genotype.

<table>
<thead>
<tr>
<th>TAXON</th>
<th>PHENOMENON</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Drosophila</em></td>
<td>Within-strain sperm successfully fertilize more eggs than between-strain sperm indicating an interaction between the genotype of the female and the genotype of the sperm</td>
<td>Zimmering &amp; Fowler 1968</td>
</tr>
<tr>
<td>melanogaster</td>
<td></td>
<td></td>
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<tr>
<td>(fruit fly)</td>
<td></td>
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</tr>
<tr>
<td><em>D.</em></td>
<td>Females discriminate against sperm carrying B²⁺4 chromosomal rearrangement. Prior exposure of the reproductive tract of the female to B²⁺4 sperm seems to enhance the ability to subsequently discriminate against sperm of this genotype.</td>
<td>Childress &amp; Hartl 1972</td>
</tr>
<tr>
<td>melanogaster</td>
<td></td>
<td></td>
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<tr>
<td><em>Tribolium</em></td>
<td>In two-male matings, Chicago black genotype male achieved a significantly higher $P_2$ value when mated to Chicago black females than with wild-type females</td>
<td>Lewis &amp; Austad 1991</td>
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<tr>
<td>castaneum</td>
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<td>(flour beetle)</td>
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<td>TAXON</td>
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</tr>
<tr>
<td>Mouse</td>
<td>In matings with heterozygous females, ( t^o )-allele haplotype sperm had a higher probability of fertilizing eggs carrying the wild-type allele than eggs with the either the ( T ) or ( t^o ) alleles.</td>
<td>Bateman 1960</td>
</tr>
<tr>
<td>Rabbit &amp;</td>
<td>Whereas all the sperm failing to enter the oviduct are coated with IgG antibody, at least 90% of the few sperm reaching the oviduct do not show IgG on their acrosomes, indicating that they are “a special population selected by the tract”</td>
<td>Cohen &amp; Werrett 1975</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>In 33% of couples exhibiting chronic “infertility” in which the husband had normal semen and no antisperm autoantibody, the female’s cervical mucus samples agglutinated her partner’s spermatozoa but not donor spermatozoa</td>
<td>Dondero et al. 1978</td>
</tr>
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</table>
1800 million sperm that are deposited, for example, in the human vagina, approximately only 300 reach the site of fertilization (Austin 1995). The sperm antigens responsible for antisperm immune infertility in humans have been identified as a small group (3-5) of sperm-surface glycoproteins (Primakoff et al. 1990). In mice, sperm antigens have been shown to induce cell-mediated immune factors that decrease sperm motility and affect embryonic development (Naz & Mehta 1989). In addition, antisperm antibodies can impair sperm function both at the level of cervical mucus penetrating ability (Bronson et al. 1987; Jager et al. 1987) and gamete interaction (Clarke et al. 1985; Mandelbaum et al. 1987; D'Almeida et al. 1989).

The ability of the immune system to distinguish between proteins that differ by only a single amino acid or even between optical isomers of the same protein (Alberts et al. 1983) makes it highly likely that, in any particular female, sperm from different males may differ in the extent to which they are perceived as non-self. Strong support for this hypothesis is provided by clinical testing of apparently infertile human couples in which the male produced normal semen with no antisperm autoantibody (Dondero et al. 1978). In one third of such couples, the female's cervical mucus samples agglutinated her partner's spermatozoa but not donor spermatozoa (Table 1.2). Birkhead et al. (1993) have proposed that female antisperm responses provide mechanisms by which females ensure that their eggs are "fertilized by the fittest sperm, or minimize the risk of being fertilized by the "worst" sperm in the population." Whereas their hypothesis posits female choice based on inherent male genetic quality, the
genetic incompatibility hypothesis presented here assumes that sperm quality is a relative characteristic which depends at least in part on the genotype of the female herself.

The fact that some cell-surface antigens present on spermatozoa are the products of loci which are critically important in embryonic development (Van Blerkom 1977) is consistent with the hypothesis that an important function of the female antisperm immune response is discrimination against genetically-incompatible sperm. In mice, for example, F9 antigen has not only been detected on spermatozoa but is also expressed by preimplantation embryos. It has been found to be associated with the abnormal development of primitive teratocarcinoma cells and is thought to be the product of the developmentally critical mouse t-locus (see Van Blerkom 1977). Since meiotic drive alleles sabotage alternative alleles during spermiogenesis, it is not surprising that genes known to exhibit haploid expression in spermatids include the best studied of the polypeptides encoded within the mouse t-complex meiotic drive region, the TCP-1 gene (Willison et al. 1988), as well as a novel gene family whose members are candidates for the t-complex responder (Tcr) locus (Schimenti et al. 1988). Although the function of the TCP-1 polypeptide remains unclear, it is thought to be correlated with transmission ratio distortion factors (Silver 1985), and it is at least possible that females could use their immune response to different forms of these sperm proteins as a mechanism for discriminating against sperm carrying drive alleles. Particularly intriguing from the point of view of cell recognition is the fact that also included in the t-complex
region is a gene encoding a polypeptide likely to facilitate the species-specific binding of sperm to eggs (Silver 1993).

There is also evidence that histocompatibility genes exhibit parent-of-origin-dependent patterns of expression, with maternal non-H2 alloantigens evident at all stages of mouse embryogenesis from the 2-cell to the 4.5-day-old blastocyst stage, but paternal antigens only becoming obvious at the 6- to 8-cell stage (Muggleton-Harris & Johnson 1976). As Van Blerkom (1977) points out, such differential expression could have a central role in establishing cell-to-cell communication within the embryo and between embryonic and maternal cells. In humans, the presence in females of circulating antisperm antibodies appears to be associated with an increased incidence of spontaneous abortion, while in female cattle, guinea pigs, mice and rabbits, immunization with sperm caused an increased incidence of postfertilization infertility resulting from preimplantation embryo mortality (reviewed in Menge 1980). Prefertilization interaction between sperm genotype and the female immune system may thus provide a reliable indicator of postfertilization complementarity of maternal and paternal genotypes.

The extent to which female mammals are able to discriminate between individual sperm produced by a single male remains controversial (see Austin 1995). Even though haploid gene expression does occur, immunocytochemical analyses have established that gene products can move through the intercellular bridges connecting spermatids developing within a common syncytium (Willison et al. 1988; Braun et al. 1989). However, these studies have
demonstrated only that gene products are able to diffuse down a concentration gradient to spermatids which completely lack a gene. They have not demonstrated that alternative forms of the same haploid-expressed gene product produced within a syncytium are mixed and shared equally between all the member spermatids (Barratt 1995). The finding that X- and Y-bearing sperm in mice exhibit pronounced variation in quantity of histocompatibility-Y (H-Y) antigen present on the sperm head strongly suggests that haploid-expressed gene products are not equally shared (reviewed in Koo et al. 1977). Whether sperm phenotype reflects haploid or diploid gene expression is, in any case, relatively unimportant from the point of view of the postcopulatory potential for females to recognize genetic incompatibility generated by the agents of intragenomic conflict considered here. Essentially all the viable sperm produced by a male heterozygous for a meiotic drive allele carry that drive allele (Lyttle 1991). Similarly, the modifications to sperm genotype caused by transposable elements and cellular endosymbionts are likely to affect all the sperm produced by a male carrying such genetic elements.

In theory, choice of sperm could enable females to minimize the risks to their reproductive success imposed by cellular endosymbionts. For example, in the presence of male killers or feminizing agents, selection on nuclear genes should favor mutations which suppress the activity of the cytoplasmic sex ratio distorters (Hurst 1991), as has been shown to occur in the isopod, Armadillidium vulgare (Juchault et al. 1993). Infected females could therefore enhance their fitness by multiple mating and cryptic choice of sperm carrying
such neutralizing nuclear alleles. In the case of Wolbachia-generated cytoplasmic incompatibility, there is evidence that the cellular endosymbiont causes changes in protein composition in the reproductive tissues of infected males (Karr 1994). Multiply-mated, uninfected females could conceivably recognize these endosymbiont effects on sperm phenotype and discriminate against fertilization by such sperm. It should be pointed out, however, that multiple mating appears to have the reverse effect in Tribolium confusum beetles (Wade & Chang 1995). In uninfected females, postcopulatory sexual selection apparently favors sperm from males infected with Wolbachia pipiens over sperm from antibiotically-cured males, even though such fertilization results in embryo inviability. However, interpretation of these results is complicated by the fact that the lower success of uninfected males’ sperm may have been due to antibiotic effects on sperm mitochondrial function. In addition, since the original female founders of the infected laboratory stock presumably became infected because they lacked the ability to respond to the cellular endosymbiont, recently-cured females derived from this stock would not be expected to exhibit a response to the bacterium.

Particularly intriguing is the evidence that female choice of sperm may occur even after sperm have penetrated eggs. In the ctenophore, Beroe ovata, egg penetration by several sperm (polyspermy) is common and can result in a delay of several hours before first cleavage. During this interval, the sperm remain immobilized at their point of entry while the egg pronucleus "... acts as if it was choosing a mate" (Carré & Sardet 1984; Carré et al. 1991). In some cases, the
egg pronucleus fuses with the first sperm pronucleus encountered, while in others it migrates back and forth between the maturation pole and as many as three sperm penetration sites before fusing with one of the sperm pronuclei. In birds and reptiles, a similar process of selection may occur: several sperm may be allowed to penetrate the egg and form sperm pronuclei but only one of these fuses with the egg pronucleus (Birkhead et al. 1993). Although infrequent, polyspermy is also known to occur in mammals (Kovacs et al. 1991; Navara et al. 1994). Further evidence that eggs discriminate between sperm of different genotypes comes from recent studies of the mechanisms underlying paternal transmission of mitochondria in crosses between closely-related species of mice. Kaneda et al. (1995) present compelling evidence that the elimination of paternal mitochondria is triggered by the egg cytoplasm recognizing species-specific, nuclearly-encoded proteins in the sperm midpiece.

A final example, involving one of the few known cases of meiotic drive in females, suggests that a general feature of vertebrate meiosis, the postponement of the second meiotic division in eggs until after fertilization, may itself be a mechanism for incompatibility avoidance. In female mice heterozygous for a meiotic drive locus on chromosome 1, chromatid segregation depends on the haplotype of the fertilizing sperm (Agulnik et al. 1993). While penetration by wild-type sperm results in strong meiotic drive, with 85% of wild-type chromatids being diverted to polar bodies, segregation normalizes to 50:50 when a drive-haplotype sperm enters the egg. This ability to modify segregation patterns in response to sperm genotype has major fitness
benefits for heterozygous females since it reduces the proportion of offspring which fail because they are homozygous for the drive allele.

(c) Reallocation of maternal Investment

Compatibility between maternal and paternal genomes is likely to be particularly critical for species in which both fertilization and embryonic development occurs within the female (e.g., mammals and viviparous arthropods such as pseudoscorpions and scorpions). For a viviparous female, the optimal sperm genotype is likely to be one which can interact with her reproductive tract without generating a strong immunological antisperm response yet, at the same time, be sufficiently different at critical recognition loci to establish the immunological détente between mother and fetus essential for normal development (Beer et al. 1982). In this connection, it has been shown that a significantly higher proportion of women experiencing repeated miscarriages shared common major histocompatibility complex (MHC) antigens with their husbands when compared to control groups, indicating that fetuses not possessing alleles distinct from their mothers may be less capable of triggering a protective blocking antibody response. Similarly, the finding that, in couples with recurrent spontaneous abortion of karyotypically-normal fetuses, there was a significantly depressed response of the female’s lymphocytes when stimulated by the respective spouse’s lymphocytes but not when stimulated by
the donor lymphocytes led to the suggestion that this resulted from a failure of the mother's cellular immune system to respond to the paternal histocompatibility antigens. This hyporesponsiveness was not detected in abortions involving karyotypically abnormal fetuses (reviewed in Beer et al. 1982). These data support the hypothesis that optimal outbreeding, a phenomenon known to occur in plant populations (reviewed in Marshall & Folsom 1991), may also be a factor favoring polyandry in animals (Stockley et al. 1993).

Polyandry provides females of viviparous species with a mechanism for reducing the cost of fertilization by incompatible sperm which is not available to females that lay eggs. By mating with several males and producing mixed paternity litters, viviparous females have the opportunity to shunt resources from genetically-defective to viable embryos. This mechanism is likely to be particularly effective if females typically produce more zygotes per litter than can survive to birth. Female mice, for example, eliminate as many as one third of their fertilized eggs without affecting total litter size (Hull 1964). Ironically, it is this capacity of viviparous females to reallocate resources which also makes them especially vulnerable to intragenomic conflict. Indeed, Haig & Graham (1991) have argued that genomic imprinting can only evolve in the context of postzygotic maternal investment and multiple paternity. A theoretical model by Hurst (1991) has also shown that redirection of nutrients to female offspring following death of male embryos is critical for the spread of cytoplasmic male killers. In addition, in meiotic drive systems, the ability of females to reallocate
resources may have the counterintuitive effect of generating selection that favors recessive lethal alleles at loci closely linked to the drive locus (Lyttle 1991; Charlesworth 1994). In the t-complex system, for example, since any sons homozygous for the distorter allele will be completely sterile, early homozygote death and reallocation can be to the benefit of both the female and the drive allele. The reproductive compensation available to t-complex heterozygous female mice may well explain why the t-allele occurs at an equilibrium frequency of 10-20% (Lyttle 1991) whereas the SD drive allele achieves only a 1-6% frequency in D. melanogaster (Lyttle 1993) which is, of course, oviparous. In essence, by salvaging some reproductive success through reallocation of resources from embryos whose development has been disrupted by selfish genetic elements, females effectively depress the impact of intragenomic conflict on individual reproductive success and increase the equilibrium frequency that can be attained by the selfish genetic element.

5. CONCLUSIONS

In this paper, I have argued that an important reason why females engage in polyandry is because, by doing so, they can most effectively deal with the risks associated with intragenomic conflict. Although any one type of selfish genetic element is probably of limited importance to the average female, taken as a whole, intragenomic conflict may pose a significant threat to female
reproductive success in many natural populations. Perhaps more importantly, while I have focused here on genetic conflicts, other mechanisms could also act to generate incompatibility. For example, higher rates of mutation in males than in females (Chang et al. 1994), the instability of hypervariable DNA (Kelly et al. 1989; Richards & Sutherland 1992; Monckton 1994), inbreeding depression (Tegelström et al. 1991; Madsen et al. 1992), outbreeding depression (Waser & Price 1991), and even perhaps the spontaneous tendency of sperm to take up foreign DNA and incorporate it in embryonic genomes (Lavitrano et al. 1991), could all add to the disruption of intrapopulation genetic compatibility. From the rates of spontaneous abortion detected in both plants (e.g., 70% in outbred angiosperms; Wiens et al. 1987) and animals (e.g., 10% to 60% in mammals; Baker & Bellis 1995), it seems likely that the cumulative toll of these phenomena on female reproductive success may be high. If sperm competition, female choice of sperm and reallocation of maternal resources can occur, there is considerable scope for females to improve their reproductive success by mating with several males. The positive relationship between polyandry and offspring production detected in several recent studies (Madsen et al. 1992; Lewis & Austad 1994; Olsson et al. 1994; Zeh 1996: chap. 3) indicates that they do.
CHAPTER 2

LAST-MALE SPERM PRECEDENCE BREAKS DOWN WHEN FEMALES MATE WITH THREE MALES

1. INTRODUCTION

In his landmark paper, Parker (1970) extended Darwin's theory of sexual selection beyond mating to the point of fertilization. When females mate promiscuously, sperm competition and cryptic female choice may play an important role in sexual selection. Parker himself emphasized insects as the most likely candidates for sperm competition because females generally possess well-developed sperm storage organs in which sperm can survive for extended periods. However, it is becoming evident that the potential for postcopulatory sexual selection exists in many taxa (Smith 1984; Ginsberg & Huck 1989; Birkhead & Hunter 1990; Marshall & Folsom 1991; Birkhead & Møller 1992; Gomendio & Roldan 1993). Sperm competition has been detected even in mammals in which sperm are short-lived and fertilization can take place only during the brief period of oestrus (Ginsberg & Huck 1989; Møller & Birkhead 1989). Similarly, in many birds previously thought to be monogamous, extrapair copulations resulting in mixed paternity are now known to occur regularly (Birkhead & Møller 1992; Dunn & Lifjeld 1994).
This growing evidence for the female reproductive tract as the ultimate arena for male competition and female choice raises important questions concerning the evolutionary significance of postcopulatory sexual selection (Eberhard 1991; Birkhead et al. 1993). What factors determine patterns of sperm utilization? Is male fertilization success a simple consequence of mating order, as has been suggested by many laboratory studies (Parker 1970, 1984; Gwynne 1984; Achmann et al. 1992; Birkhead & Møller 1992)? In nature, are sperm precedence patterns actually more complex, but still predictable functions of other prior-stage factors in addition to mating order, such as quantity of sperm or timing of copulation (see Birkhead et al. 1988)? Alternatively, is sexual selection within the female a distinct process in which copulatory courtship (Eberhard 1991) and/or attributes of sperm themselves (Sivinski 1984; Birkhead et al. 1993) can override the effects of selection acting at earlier stages? Resolution of these questions promises to provide insights into the impact of polyandry on the operation of sexual selection and is likely to have major implications for the criteria used to estimate male reproductive success in natural populations.

Until recently, attempts to document patterns of sperm precedence have been constrained by the methods available for discriminating between sires. Paternity assignment based on the irradiated male technique or electrophoretic or phenotypic markers has largely restricted investigations to two-male mating designs (Burke 1989). Such studies on insects and birds have generally found a strong mating order effect in which the last male to mate fertilized the majority
of eggs (Parker 1970, 1984; Gwynne 1984; Ridley 1989; Birkhead & Møller 1992). However, these results may not accurately reflect how females utilize sperm in nature (Martyniuk & Jaenike 1982; Ginsberg & Huck 1989; Schwartz et al. 1989; Oxford 1993). In the wild, females of many species mate with more than two males (Eberhard 1985). In addition, it has recently been shown that the capacity of females to fertilize a succession of egg batches with sperm stored over extended periods can significantly undermine any initial mating order effect (Siva-Jothy & Tsubaki 1989).

With the development of single-locus DNA profiling methods (Armour et al. 1990; Queller et al. 1993), hypervariable micro- or minisatellite regions can now be cloned for use as powerful genetic markers capable of discriminating between several putative sires. Locus-specific banding patterns are easy to score and clearly reveal allelic inheritance patterns (see Fig. 2.1). The extreme variability inherent in certain minisatellite loci (heterozygosities of 95-99%) can also be exploited as a means of estimating the extent of multiple paternity in natural populations. Simply tallying the number of non-maternal alleles provides a sensitive measure of the minimum number of males involved in fertilizing a brood.

The use of recently developed single-locus minisatellite probes (Zeh et al. 1994) has unexpectedly revealed high levels of multiple paternity in the harlequin beetle-riding pseudoscorpion, Cordylochernes scorpioides (Zeh & Zeh 1996a.). This pseudoscorpion has an intriguing natural history in which males defend harlequin beetle abdomens as strategic sites for intercepting and
Fig. 2.1. An example of paternity assignment, using the probe CscMS13. For this three-male replication, a total of 32 offspring were fingerprinted on two gels. Overall, male A sired 16%, male B 65% and male C 19% of the offspring. All four parents were heterozygotes and possessed distinct alleles. However, because there was less than 1 mm migration distance between male A's higher molecular weight allele and male C's lower weight allele, I did not distinguish between these two alleles (band E). A second probe (CscMS23) was therefore used to confirm the paternity of offspring 15 and 17 (gel not shown).
inseminating dispersing females (Zeh & Zeh 1994c). In a sample of 23 females naturally dispersing on beetles, 16 produced some offspring sired by attendant beetle-riding males (assumed to be the last males to mate, see Zeh & Zeh 1992, 1994a). However, the majority of females (74%) produced mixed-paternity broods with no evidence of last-male sperm precedence. In one set of 10 offspring, sperm from at least four males fertilized the brood (Zeh & Zeh 1996a).

Clearly, the high incidence of multiple paternity in these field results for C. scorpioides is not consistent with the strong last-male sperm precedence pattern typical of two-male mating experiments performed on other terrestrial arthropods, primarily insects. Neither is it consistent with the first-male sperm precedence detected in some arachnids, notably spiders (Austad 1984; Watson 1988). Are pseudoscorpions therefore aberrant in their utilization of sperm or do other factors account for the discrepancy? In this study, I have addressed this question by carrying out a sperm precedence experiment on C. scorpioides in which I experimentally manipulated both the number of males mated to a female and the time interval between matings, and used single-locus DNA profiling to assign paternity.

2. METHODS

(a) Sperm precedence experiment and sperm counting
Pseudoscorpions were collected between October and December 1992 from three decaying fig tree (*Ficus sp.*) populations in the lowland rain forest of Soberania National Park, Republic of Panama (see Zeh & Zeh 1992). Virgin females (*n* = 44) were obtained by collecting immatures and maintaining them in individual vials until they molted to adults. Males (*n* = 93) were collected either as adults or final stage immatures (tritonymphs).

The experimental design consisted of three treatments: 1, two-male mating with 24 h between matings; 2, two-male, 96 h interval; 3, three-male, 24 h interval. Each replication was initiated by placing a virgin female with a male (male A) in a 28 mm-diameter mating arena. Interactions were videotaped (Panasonic PV-S350 S-VHS camcorder) for approximately 45 min under red, fibre optics illumination. The videotape was then transcribed to determine whether mating had occurred. After the appropriate time lapse, the procedure was repeated with a second (male B) and, where appropriate, a third male (male C).

In these pseudoscorpions, successful mating involves a well-defined sequence of behaviors in which the male grasps the female while he deposits a stalked spermatophore on the substrate. At the spermatophore's apex is a complex, folded, tubular packet containing the sperm (Weygoldt 1969; Zeh & Zeh 1994a). After spermatophore deposition, the male maneuvers the female into a position where the sperm packet directly contacts her genital aperture. High magnification video analysis (Panasonic CCTV WV-1410 camera equipped with a 55 mm Micro- NIKKOR lens and two Nikon PK-13 extension
tubes) has revealed that successful attachment of the sperm packet to the
gonopore is associated with a pronounced abdominal flexure by the female.

This combination of external spermatophore deposition and diagnostic
female behavior provides a unique, non-invasive window on mating event
characteristics such as the number of spermatophores accepted and rejected
by a female. In this experiment, I discarded any replications involving
ambiguous sperm packet transfer. The reproductive status of the remaining
females was monitored for approximately two months. Females carry batches of
developing embryos in an external brood sac and monitoring the females was
therefore possible with minimal disturbance. From each female, two broods
were collected after nymphal hatching and reared to adults. Mothers, putative
sires and offspring were frozen at -70°C.

For a subset of the replications, I obtained a post-experimental estimate of
the number of sperm transferred by each male (n = 36 males). This was carried
out by placing the male (after a two-day lapse and supply of food) with a
non-experimental female under a stereomicroscope (20X magnification), and
interrupting the mating immediately after spermatophore deposition. The sperm
packet was collected by adhesion to a dissecting needle and broken up in 10 μl
of a 10% solution of Tween 80 (FisherScientific) to disperse the encysted
sperm. A hemocytometer was used to count sperm, which are visible without
staining, under a compound microscope at 200X magnification.

For nine of the 36 males, I was able to assess whether sperm number was
constant across females and through time. In each of these cases, the male had
produced two spermatophores during the experimental mating itself, the second of which had been refused by the female. The rejected spermatophore had remained intact enabling the packet to be collected after the mating and the sperm to be counted. The mean sperm number (± s.e.) for these rejected spermatophores (1,069 ± 106) did not differ significantly (p > 0.05) from that of spermatophores produced with the non-experimental females (1,228 ± 68). Moreover, there was a significant, positive correlation between these two measures of a male's sperm number (r = 0.65, p = 0.03, one-tailed).

(b) DNA profiling

DNA profiling was carried out as described elsewhere, using two hypervariable single-locus minisatellite probes (CscMS13 and CscMS23) cloned from a genomic library of *C. scorpoides* (Zeh et al. 1994). Briefly, genomic DNA was isolated by grinding whole adults in 400 μl of 2X CTAB buffer and performing two chloroform and three phenol/chloroform extractions (Zeh et al. 1992a). For each sample, one third of the extracted DNA was retained (see below). The remaining two-thirds (2.5 μg) was digested for 10 h with four-fold excess of *HaeIII* or *Mbol* and run on a 1% agarose gel for 36 h at 36 V in circulating TBE buffer. Size-fractionated DNA was capillary blotted and fixed onto nylon membranes (Zetabind, Cuno Inc.) by baking for 3 h at 80°C. Hybridization was carried out using double-stranded, gel-isolated probe inserts
random prime-labelled with $^{32}$P. Membranes were hybridized at 63°C in phosphate buffer (Westneat et al. 1988) and washed for 30 min in 2X SSC, 0.1% SDS at 25°C and again at 65°C.

For each replication, DNAs from the mother, putative sires and approximately 25 offspring from the two broods were run on a single gel. For three-male replications, 10-15 additional offspring were run on a second gel with the remaining DNA of the parents. The highly variable nature of these minisatellite loci (heterozygosities ≥ 0.95; see Zeh et al. 1994) made it possible to assign paternity by simple visual comparison of offspring and putative paternal bands (Fig. 2.1).

3. RESULTS

Of the 44 original females, 14 were excluded at the mating stage either because they refused to accept spermatophores from all males involved in the replication, or because videotape transcription left some doubt that successful sperm transfer with all males had occurred. Two females failed to become gravid and two produced brood sacs which aborted early in development. For the remaining 26 females, two broods of offspring were collected after nymphal hatching and reared to adults. The mean interval between hatching of the first and second brood was 27 da. The mean number of nymphs hatched 57 for the
first brood one and 56 for the second. For paternity assignment, I opted to fingerprint a large number of offspring per female. While this restricted my profiling to 14 replications, it was necessary in order to provide a representative sample of each female's broods, which varied in number between 30 and 80 protonymphs. This approach also increased the likelihood of detecting subtle between-treatment differences as well as within-female, between-brood differences in patterns of sperm precedence. In all, 404 offspring were fingerprinted.

The two-male, 24 h treatment resulted in essentially complete last-male sperm precedence. Across the five replications, 135 of 137 offspring were sired by male B (mean ± s.e. = 98% ± 2%). However, this pattern proved not to be robust to change in either the time between matings or the number of males involved (Fig. 2.2). When the time lapse was extended to 96 h \( (n = 5) \), the proportion of offspring sired by male B became much more variable, ranging from 54% to 100% with a mean of 75% ± 8% (104 of 136 offspring). When the interval between matings was held at 24 h but the number of males was increased to three \( (n = 4) \), the pattern of last-male sperm precedence broke down completely. In this treatment which involved a total of 141 offspring, male C actually sired the lowest mean proportion of offspring: 21% ± 8% compared to 34% ± 7% for male A and 45% ± 9% for male B. None of these percentages is significantly different from 33%, i.e., there was no mating order effect in the three-male treatment.

To test the statistical significance of these differences in patterns of sperm
Fig. 2. 2. Summary of the proportion of offspring sired by each male (mean ± s.e.) in each of three mating treatments.
precedence between mating treatments, I carried out a one-way analysis of variance with the proportion of offspring sired by the last male to mate \( (P_{\text{last}}) \) as the dependent variable. Treatment effect accounted for 81% of the overall variance in \( P_{\text{last}} \) and was highly statistically significant (one-way analysis of variance on arcsin-transformed data, \( F = 23.98, 13 \) d.f., \( p < 0.0001 \)). Multiple comparison tests (Bonferroni, Scheffe and Tukey's Studentized Range, SAS 1988) showed significant difference for all three pairwise comparisons of means (\( p < 0.05 \)).

In contrast to the strong effect of mating design on sperm precedence, patterns of paternity generally did not differ between first and second broods of a female. Of the eight replications exhibiting some level of mixed paternity, in only one (a three-male replication) did the relative reproductive success of males vary significantly between broods (Fisher's Exact Test, \( p = 0.05, n = 42 \) offspring). In this case, the proportion of offspring sired by male A dropped from 47.62% (10 of 21) in brood 1 to 14.29% (3 of 21) in brood 2. This was counterbalanced by an increase for male B from 23.18% to 52.38%. Brood effect, however, was not significant when corrected for the number of tests (Bonferroni critical \( p = 0.006 \), see Rice 1989).

Variation in mating order effects among the three treatments necessitated different approaches to examining the relationship between fertilization success and the proportion of sperm transferred by a male. Data from the two-male, 24-h treatment were not analyzed since the overriding influence of last-male sperm
precedence obviously precluded the possibility of any effect of sperm number. Because mating order exerted some effect in the two-male, 96-h treatment, I assessed whether variation in $P_{\text{last}}$ was attributable to variation in the proportion of sperm transferred by male B. Interestingly, second males contributing more sperm (relative to the first male) tended to sire a lower proportion of offspring than second males contributing less sperm. However, the relationship was not significant ($r = -0.44$, $n = 4$, $p > 0.05$), perhaps because of the small sample size. In the three-male treatment, where mating order could be ignored, the estimated proportion of sperm contributed by a male was not significantly correlated with the proportion of offspring sired ($r = 0.28$, $p > 0.10$).

4. DISCUSSION

The existence of multiple paternity in the harlequin beetle-riding pseudoscorpion is of general interest because it represents a striking departure from the pattern of strong last-male sperm precedence apparently pervasive among other terrestrial arthropods (Birkhead & Hunter 1990). My results indicate that shared paternity in *C. scorpioides* is not the consequence of an inherent absence of mating order effects in this pseudoscorpion, but instead depends critically on the mating context in which sperm utilization occurs.

By manipulating the number of males or the interval between matings, I was able to induce three significantly different patterns of sperm utilization. When
females were mated to two males 24 h apart, the last male to mate sired essentially all the offspring. Extending the time lapse in two-male matings significantly reduced but did not eradicate the second-male advantage. By contrast, addition of a third male resulted in highly mixed paternity and complete elimination of mating order effects.

In a number of arachnids, including pseudoscorpions, the transfer of sperm indirectly via externally-deposited spermatophores is associated with encysted sperm which must de-encyst within the female's reproductive tract (Baccetti 1979; Legg 1973). The weakening of last-male sperm precedence in the two-male, 96 h treatment may have resulted primarily from the first males' sperm partially overcoming their mating order disadvantage by earlier de-encystment. Interestingly, increasing the interval between matings has been found to have the opposite effect in birds (e.g., Birkhead et al. 1988).

Two main mechanisms have been proposed to account for last-male sperm precedence (Birkhead & Hunter 1990). These are 1) stratification of sperm and 2) sperm displacement/removal. In C. scorpioides, the fact that reproductive success was shared by all males in three-male replications strongly argues against sperm displacement. It seems likely that the last-male sperm precedence effect observed in both of the two-male treatments resulted from sperm stratification within the female's paired, tubular, blind-ending spermathecae. Walker (1980) proposed that the anatomy of female sperm storage organs should influence patterns of sperm precedence, with tubular spermathecae favoring last males via stratification and spherical shapes
permitting mixing of males' sperm. However, Ridley (1989) in an extensive review found little empirical support for this hypothesis.

I suggest that, although tubular spermathecae may favor sperm stratification, this relationship breaks down if spermathecae are filled to capacity, thereby generating sufficient pressure to cause sperm mixing. In the harlequin beetle-riding pseudoscorpion, females usually accept only a single spermatophore per mating event (mean ± s.e. = 1.18 ± 0.05, n = 65 matings). Thus, in the two-male treatments there may have been inadequate sperm transferred to fill the spermathecae. Sperm from male A may therefore have been shunted to the blind end of the spermathecae by male B's sperm. This proposed mechanism, which remains to be tested, would explain the otherwise perplexing trend in the two-male, 96 h treatment for second males transferring fewer sperm to achieve higher reproductive success. Under this scenario, the transfer of a large number of sperm by male B would be more likely to fill the spermathecae and cause sperm mixing, thereby enabling male A to achieve fertilization success. In the three-male treatment, the transfer of an additional batch of sperm by a third male would precipitate sperm mixing and multiple paternity to an even greater extent.

The degree to which previous studies have been dominated by the two-male mating design is amply illustrated by the fact that the proportion of offspring fathered by the last male to mate has come to be known as the $P_2$-value. The high $P_2$-values in many of these studies have been taken as evidence of last-
male sperm precedence in nature. In certain extreme cases, this interpretation seems quite justified, as in Odonata (Waage 1984; Siva-Jothy & Tsubaki 1989). In some damselflies and dragonflies, males remove previous male's sperm from the female's reproductive tract before mating. However, in many other species, the implicit assumption that $P_2$ is largely independent of the number of males with whom a female mates may not be valid: in $C. scorpioides$ the last-male advantage broke down completely when females were mated to three males. Although the generality of my findings remains to be determined by similar studies on other species, my data clearly cast doubt on the validity of extrapolating sperm precedence patterns based on simple laboratory experiments to field populations. Moreover, if females can override mating order constraints on sperm utilization by mating with several males, the opportunity for postcopulatory sexual selection through sperm competition and/or cryptic female choice may be much greater in nature than is evident from two-male mating experiments.
CHAPTER 3

DIMINISHING THE IMPACT OF GENETIC INCOMPATIBILITY: POLYANDRY
AND ENHANCED REPRODUCTIVE SUCCESS IN THE BEETLE-RIDING
PSEUDOSCORPION

1. INTRODUCTION

Molecular genetic approaches to behavioral ecology are increasingly demonstrating that multiple paternity, and hence polyandry, is a widespread feature of natural populations (e.g., Schwartz et al. 1989; Birkhead & Møller 1992; Amos et al. 1993; Schultze-Hagan et al. 1993; Dunn & Lifjeld 1994; Schenk & Kovacs 1995). Identifying the reasons why females of so many species mate with several males is of fundamental importance to the study of sexual selection (Gowaty 1994) and polyandry is therefore currently the subject of much debate (Madsen et al. 1992; Parker 1992; Capula & Luiselli 1994; Keller 1994; Olsson et al 1994). However, the diversity of hypotheses to explain this behavior and the technical difficulties associated with evaluating these alternative hypotheses make investigation of female polyandry largely intractable in many species. With the exception of polyandry resulting from forced copulation (Thornhill 1980), current hypotheses can be classified broadly according to whether they propose material benefits or some form of genetic
benefit (e.g., Simmons 1992; Loman et al. 1988; Birkhead et al. 1993). As
Parker (1992) has pointed out, although polyandrous mating for material
benefits is supported by direct evidence, the argument that females gain genetic
benefits by mating with several males is extremely controversial. Perhaps the
strongest support for genetic benefits comes from a field study of adders in
which stillborn offspring production was significantly reduced when females
mated with several males (Madsen et al. 1992). In this study and similar
research carried out on sand lizards (Olsson et al. 1994), the authors concluded
that, by generating sperm competition, multiple mating results in a higher
proportion of eggs being fertilized by genetically-superior males.

Implicit in this emphasis on intrinsic male quality is the premise that the
nuclear and cytoplasmic genomes of any female in a population can be
combined with the nuclear genome of any male to produce a viable zygote
which can complete embryonic development and attain sexual maturity. This
assumption seems increasingly unrealistic. It is becoming evident that the
nuclear and cytoplasmic genomes of species are not at evolutionary equilibrium
(Hurst 1992) but are dynamic entities in which meiotic drive (Lyttle 1991),
hypervariable DNA (Charlesworth et al. 1994), cellular endosymbionts (Hurst
1993), genomic imprinting (Haig 1993) and accelerated rates of mutation
(Chang et al. 1994) and endosymbiont infection in males (Stevens & Wade
1990) can all undermine the reproductive cohesiveness of a population (see
chap. 1). Sexual reproduction thus involves the merging in embryos of maternal
and paternal genomes which are likely to vary in the extent to which they are
genetically compatible. Elsewhere, I have proposed that females may engage in polyandry because, by accepting sperm from several males, they can exploit postcopulatory mechanisms (sperm competition, female choice of sperm and reallocation of maternal investment) in order to minimize the risks and or costs of fertilization by genetically-incompatible sperm (see Chapter 1).

The recent development of single-locus minisatellite DNA probes for the harlequin beetle-riding pseudoscorpion, *Cordylocnemes scrophiodes* (Zeh et al. 1994), in combination with a reproductive biology highly amenable to experimentation and involving the production of large numbers of offspring (Zeh & Zeh 1994a), makes this species a model system for investigating the causes and consequences of female polyandry. Fundamental to the hypothesis that females mate with multiple males to diminish the risk of genetic incompatibility is the prerequisite that sperm utilization in nature should be free of the strong mating order effects detected in many two-male sperm precedence experiments across a wide range of species (Birkhead & Hunter 1990). In field populations, *C. scrophiodes* females are polyandrous and produce broods sired by as many as four males (based on a mean of 7 offspring fingerprinted per brood, Zeh & Zeh 1994b). A sperm precedence experiment carried out to investigate this apparently unusual absence of mating order effects demonstrated that last-male sperm precedence can be induced in this pseudoscorpion by mating females to two males within 24 h. However, the last-male advantage was markedly reduced by increasing the interval between matings and broke down completely when females were allowed to mate with three males (see Chapter
2). This elimination of mating order constraints on sperm utilization when females mate with several males suggests that the opportunity for postcopulatory sexual selection may be much greater in nature than is evident from two-male mating experiments.

In this chapter, I present the results of a study which shows that female polyandry in *C. scorpoides* is a deliberate mating strategy and that it increases female reproductive success primarily by reducing the rate of embryo failure. This study differs from other investigations of the adaptive significance of polyandry (Madsen et al. 1992; Olsson et al. 1994) in two important ways. First, it was carried out on individuals from large, outbred populations as opposed to small, marginal populations in which the risk of inbreeding may exert an atypically strong effect on female mating tactics and reproductive success. Second, it is the first investigation designed not only to test the material versus genetic benefits hypotheses in an animal system but also to differentiate between inherent male genetic quality and genetic compatibility as a cause of the higher reproductive success of polyandrous females. By eliminating inherent male quality and other previously proposed hypotheses, this research points to the threat of genetic incompatibility as a force driving the evolution of multiple mating by females.
2. MATERIALS AND METHODS

(a) Natural history and reproductive behavior of *C. scorpionoides*

This neotropical pseudoscorpion inhabits decaying trees in the families Moraceae and Apocynaceae (Zeh & Zeh 1992, 1994a). The larval wood-boring activity of its dispersal agent, the harlequin beetle, *Acrocinus longimanus*, creates a microhabitat of exfoliating bark and accumulated sawdust (Zeh et al. 1992b) which supports a diverse community of terrestrial arthropods (Zeh & Zeh 1994c). Like all pseudoscorpions in the family Cheremetidae, *C. scorpionoides* possesses only rudimentary eye spots and interactions between conspecifics are mediated largely via surface vibrations and direct physical contact (Chamberlin 1931; Weygoldt 1969). Males appear unable to recognize females at a distance and attempt to initiate mating with any individual encountered by forcefully grasping its chelae (Zeh 1987; Zeh and Zeh 1996). If the individual is another male, the outcome is combat (Weygoldt 1969; Zeh 1987).

Mating involves a well-defined sequence of behaviors in which the male holds the female in a stationary position while he assembles and deposits a stalked spermatophore on the substrate (Zeh & Zeh 1994a,b). At the apex of the spermatophore is a complex, folded, tubular packet containing the sperm (Zeh & Zeh 1994a). After spermatophore deposition, the male reverses and attempts to pull the female over the spermatophore so that the sperm packet directly contacts her genital aperture. High magnification video analysis (Panasonic
CCTV WV-1410 camera equipped with a 55 mm Micro-Nikkor lens and two Nikon PK-13 extension tubes) has revealed that successful attachment of the sperm packet to the gonopore is associated with a pronounced abdominal flexure by the female (see Chapter 2). This action causes a long, hooked tube to evert from the packet into the female's genital aperture, followed by evacuation of sperm into her reproductive tract. A lapse of approximately 15 min is required between successive spermatophore depositions, during which the male attempts to maintain his hold on the female (unpubl. data).

Female pseudoscorpions possess sperm storage organs (spermathecae; see Legg 1975) and can produce at least four clutches of offspring from a single insemination (unpubl. data). After becoming gravid, females use sawdust and silken threads to construct a brood nest in which they remain until 2 to 3 days after the nymphs hatch. Embryos are communally nourished through ovarian secretions by the female in a translucent, external brood sac overlying her genital aperture. Nymphs hatch after approximately 14 days and remain with their mother in the brood nest until they become fully sclerotized.

(b) General methods

The study consisted of: (1) video analysis of mating behavior to define polyandry in C. scorpoides; (2) mating and rearing experiments to compare patterns of offspring production in three categories of C. scorpoides females:
females restricted to mating with one male in the laboratory; females mated to two or three males in the laboratory, and field-inseminated females, and (3) a comparison of offspring production by pairs of females sharing a common mate to assess the effect of male quality on female reproductive success.

The pseudoscorpions were collected from field populations known from both multilocus (Zeh et al. 1992a) and single-locus (Zeh et al. 1994) minisatellite DNA fingerprinting to be outbred and highly genetically variable at the marker loci. Laboratory-mated females were collected as nymphs from decaying fig trees (Ficus spp.) in the lowland rain forest of Soberania National Park, Republic of Panama (see Zeh & Zeh 1992), and were then reared individually to virgin adults in the laboratory. Field-inseminated females were collected from harlequin beetles and decaying trees in Panama and French Guiana (see Zeh et al. 1992b for location), as well as in forest bordering the Blanchisseuse Road in north-central Trinidad. Statistical analyses were carried out using SAS (SAS 1988).

(c) Female mating behavior

The combination of external spermatophore deposition by males and diagnostic sperm uptake behavior by females greatly facilitates non-invasive verification of successful sperm transfer and characterization of patterns of female receptivity. Matings \( n = 205 \) were carried out in a darkened room by
placing a female with a randomly-selected male in a 28 mm-diameter mating arena and videotaping interactions (Panasonic PV-S350 S-VHS camcorder) for 45 min under red, fiber-optics illumination. The videotape was then transcribed to determine the number of spermatophores deposited by the male and the number of sperm packets accepted by the female. The procedure was repeated for a subset of these females ($n = 55$) which were placed with a second male between 24 and 48 hr after their first mating.

\[\text{(d) Effect of number of mates on female reproductive success}\]

To assess the effect of mating with more than one male on female reproductive success, I compared the mean number of nymphs hatched by females mated to two or three males ($n = 27$) with that of females mated to a single male in the laboratory ($n = 199$). The high variance in the reproductive success of females mated to a single male necessitated a large-scale mating and rearing program conducted over a period of approximately two years. Each block of approximately 50 females spanned 4 months, the time required to carry out all the matings and monitor females through brood sac development to nymph hatching. One block of this treatment ($n = 50$ females) was carried out simultaneously with the multi-male mating treatment. Laboratory values were then compared to nymphal hatching counts for females naturally-inseminated in the field ($n = 56$). This latter category provided a field multiply-mated female
control group: DNA fingerprinting evidence of mixed paternity broods has established that when *C. scorpioides* females are collected as adults from natural populations, at least 75% have mated with two or more males (Zeh & Zeh 1996b). Females were maintained individually in 12 dram, plastic, transparent vials containing naturally-produced *Ficus insipida* sawdust and were fed 10-12 termite workers (*Nasutitermes sp.*) per week. The reproductive condition of all females was closely monitored until nymphs hatched from the brood sac. Since embryonic development within the translucent brood sac is readily visible with the naked eye and females generally constructed their brood nests on the walls of their vials, it was possible to monitor female reproductive status with minimal disturbance. After nymphs had hatched from the female’s brood sac and shortly before they had completed sclerotization, brood nests were carefully removed from the vial and the nymphs were counted.

*(e) Effect of male quality on female reproductive success*

The hypothesis that females mate with several males in order to increase the probability that their eggs will be fertilized by intrinsically superior males (Birkhead et al. 1993) would predict that the reproductive consequences of mating with any particular male should be consistent across females. To test this prediction, males (*n* = 67) were each mated to a randomly-selected female and then provided with a supply of termites before being mated to a second
female after a lapse of at least two days. The reproductive condition of females was closely monitored and nymphs were counted as described above. To factor out the effect of female size on nymph production, residuals were calculated from a linear regression of hatched protonymph number on female cephalothorax length. Measurements were computed from high magnification (approximately 30X) video images loaded into NIH Image (version 1.58) running on a Power Macintosh 7100AV personal computer.

3. RESULTS

(a) Female mating behavior

Virgin females were almost invariably receptive, with only 1% refusing to accept any sperm packets. However, although males often produced two and sometimes three successive spermatophores (see Fig. 3.1), 88% of the remaining 203 females refused to pick up more than one sperm packet per mating event. In the subset of females placed with a second male 24 to 48 hr after their first mating, 85% accepted a sperm packet from this male. Once again, almost all these females refused to accept more than one sperm packet (Fig. 3.1). It was evident from the videotaped matings that female cooperation was essential for successful sperm transfer. In some cases, females terminated mating by breaking free from the male's grasp before he initiated construction of
Fig. 3.1. Frequency distribution of the number of spermatophores accepted by females in their first (upper) and second (lower) mating. The second mating was carried out 24 to 48 hr after the first mating. Hatched area indicates the number of matings in which the male deposited a subsequent spermatophore which was rejected by the female.
a second spermatophore. Alternatively, females occasionally refused to remain stationary during spermatophore deposition, forcing the male to move and lose contact with the still incomplete spermatophore. Finally, in the majority of cases (approximately 70% of females accepting a single spermatophore), females cooperated with males throughout the entire period of spermatophore construction and deposition, only to resist being pulled forward over the sperm packet. Males thwarted at this final stage appeared locked into a stereotypical behavior. Forced to release their grasp on the female, they continued to reverse with their pedipalps convulsed by spasmodic jerking movements.

(b) Effect of number of mates on female reproductive success

When cases of complete brood failure were included in the analyses, the data on number of nymphs hatched were not normally distributed (Fig. 3.2). Statistical analyses were therefore carried out on ranked data. For females mated to a single male, analysis of variance (ANOVA) detected no evidence of block effects ($F_{3,198} = 0.98, P = 0.4012$) on female reproductive success and data were therefore pooled for this category of females. Similarly, in the case of field-inseminated females, geographic origin of population was found to have no effect on mean number of nymphs hatched ($F_{2,55} = 0.02, p = 0.9837$) and data were again pooled. An overall nonparametric ANOVA (i.e., an ANOVA
Fig. 3.2. Frequency distribution of the number of nymphs hatched by females mated to a single male in the laboratory (upper), by females mated to multiple males in the laboratory (center) and by field-inseminated females (lower).
carried out on ranked data) showed a significant effect of mating category on female reproductive success ($F_{2,280} = 10.17, \ P < 0.0001$; see Fig. 3.3). Females mated to multiple males in laboratory produced an average of 51.41 nymphs (s.d. = 19.08). This value did not differ significantly from the mean of 49.32 nymphs (s.d. = 21.58) hatched by field-inseminated females ($P > 0.05$, Tukey's studentized range). By contrast, females mated to a single male produced significantly fewer nymphs (mean = 35.19, s.d. = 26.50, $P < 0.05$, Tukey's studentized range). The contrast (Rosenthal & Rosnow 1985) between laboratory, singly-mated females versus field-inseminated and laboratory, multiply-mated females was highly significant ($F = 19.76, \ p < 0.0001$). The lower reproductive success of singly-mated females was not improved by accepting more than one sperm packet: the few females that did accept two packets from a single male did not produce more nymphs (mean = 38.79, s.d. = 35.03, $n = 14$) than females accepting only one (mean = 39.61, s.d. = 24.8, $n = 137$; $t = 0.67; \ P = 0.50$; square-root transformed data). The lower mean and greater variance in brood size of females mated to one male was found to result primarily from a rate of complete brood failure higher than that suffered by the two other categories of females. Broods were recorded as having failed when previously gravid females were found to no longer be carrying a brood sac. Entire broods failed to complete embryonic development in 27% of females mated to a single male, compared to only 7% of multiply-mated females and 9% of females inseminated in the field ($P = 0.002$, Fisher exact test). When cases of complete brood failure were removed from the analysis, there was still a significant,
Fig. 3.3. Comparison of mean number of protonymphs (± s.e.m.) produced by laboratory singly-mated females (broken down by blocks 1-4), laboratory multiply-mated females and field-inseminated females (broken down by source population: FG = French Guiana; PAN = Panama; TN = Trinidad).
overall effect of mating category on number of nymphs hatched \( (F_{2,220} = 4.10, \ p = 0.0178) \). Again, the contrast between laboratory, singly-mated females \( (48.16\pm18.34) \) versus laboratory, multiply-mated (mean ± s.d. = 55.52±12.51) and field-inseminated females \( (54.16\pm15.67) \) was significant \( (F = 8.00, \ p = 0.0051) \).

(c) *Effect of male quality on female reproductive success*

The numbers of nymphs produced by each male's two mates were found to be completely uncorrelated \( (r_s = -0.065; \ P > 0.05; \text{see Fig. 3.4}) \) based on Spearman correlation analysis of residuals from a linear regression of hatched protonymph number on female cephalothorax length \( (r = 0.251, \ P = 0.004) \). The absence of a consistent effect of intrinsic male quality was most apparent in the 29 cases of complete brood failure (Fig. 3.4). Broods of both females failed for only two of the 67 males. However, for 25 of the remaining males, mating resulted in complete brood failure in one female but successful nymph hatching in the other.
Fig. 3.4. Relationship between the number of nymphs hatched by female #1 and female #2 when males were each mated to two females. The absence of any significant correlation \((r = -0.065; P > 0.05)\) demonstrates that female reproductive success was not affected in a consistent way by intrinsic male quality. Here I present nymph hatching data uncorrected for female size in order to more clearly illustrate instances of entire brood failure (see text).
4. DISCUSSION

This study provides evidence that polyandry in *C. scorpioides* is a deliberate female mating strategy. Although males often produced three successive spermatophores during a single mating event, the vast majority of virgin females refused to accept more than one sperm packet. However, when these females were subsequently placed with a second male, most were again receptive. As in their first mating, they accepted only one sperm packet. Under natural conditions, males and females dissociate after copulation (Weygoldt 1969). As a consequence, this tactic of staggering sperm collection across matings is likely to ensure that most females accumulate sperm from several different males. This behavior would explain the high level of mixed paternity detected in field populations (Zeh & Zeh 1994b). My results indicate that polyandry is an adaptive behavior that increases reproductive success. Females randomly mated to multiple males in the laboratory produced as many nymphs as females having the opportunity to mate freely in the field. By contrast, females restricted to mating with a single male in the laboratory hatched significantly fewer nymphs. Although successful broods of these singly-mated females tended to be smaller (fewer hatched nymphs) than those of females mated to multiple males, this difference was not statistically significant. The lower reproductive success of the single-male category was, in fact, largely attributable to the higher rate of entire brood failure experienced by these females. Results of the experiment in which males were each mated to two females strongly suggest
that such reproductive failure is not simply the consequence of mating with an inherently inferior male. The complete absence of any correlation between the numbers of nymphs produced by each male's two mates was particularly striking in those cases in which the entire brood of one female failed while the other successfully hatched nymphs. It is extremely unlikely that brood failure resulted from inbreeding since the pseudoscorpions used in this study were derived from several different tree populations and tree populations are known to be highly genetically variable (Zeh et al. 1994).

It might be argued that the females mated to one male had lower reproductive success because they were deprived of the opportunity to choose their mate. This was the conclusion reached by Partridge (1980), based on a study of Drosophila melanogaster in which the offspring of females mated to a single, randomly-selected male exhibited lower larval survivorship than the offspring of females given the opportunity for mate choice. The study by Partridge (1980), however, was not designed to distinguish between female choice and male competition. Direct male competition might therefore have been an important factor determining which males mated with the `choosing' females (Andersson 1994:200). In addition, since females were allowed to choose mates simply by placing them in cages with many males, these females were also provided with the option to mate with more than one male. This important difference between treatments in the opportunity for postcopulatory sexual selection through sperm competition or female choice of sperm was not considered. Subsequent attempts to replicate this experiment have either failed
to produce the same result (Schaeffer et al. 1984) or have again been unable to establish whether female choice or male competition was responsible for the outcome (Taylor et al. 1987). In the case of *C. scorpionides*, mate choice can be excluded as a cause of the higher reproductive success of females multiply-mated in the laboratory. Since these females were mated sequentially to individual, randomly-selected males, they were afforded no more opportunity for mate choice than females mated to a single male. Indeed, the absence of any significant difference between the number of nymphs produced by these laboratory multiply-mated females deprived of choice and that of females allowed to mate freely in the field suggests that mate choice may not be an important mechanism of sexual selection in this pseudoscorpion.

The lower rate of embryo failure experienced by polyandrous *C. scorpionides* females cannot be accounted for by hypotheses previously proposed to explain why females mate multiply despite potentially high costs (Chapman et al. 1995; Keller & Reeve 1995). These hypotheses include forced copulation (Thornhill 1980), insufficient sperm from a single mating (Ridley 1988), male nutrient donations from spermatophores or seminal fluid (Simmons 1992) and genetic benefits deriving from intrinsic male quality and sperm competitive ability (Keller & Reeve 1995; Birkhead et al. 1993). Clearly, forced copulation is not possible in *C. scorpionides*. The indirect method of sperm transfer utilized by this pseudoscorpion not only requires precise maneuvering and female cooperation, but also affords females ample opportunity to refuse sperm while minimizing the risk of injury by feigning acquiescence. Indeed, even if receptive,
females occasionally modify their usual sequence of behaviors in a way which suggests that they are actively promoting unpredictability as a means of undermining males’ ability to assess their receptiveness. In approximately 10% of laboratory matings, the female resists the male’s efforts to pull her over the first spermatophore deposited. However, once the male has released his grasp and is locked in pedipalpal spasms, the female then moves forward and picks up the sperm packet on her own. Thus, from the male’s standpoint, it is not certain that resistance by the female even at this late stage actually translates into rejection of the spermatophore. This uncertainty may have the effect of restraining male attempts to forcefully coerce females into accepting sperm.

My results indicate that it is unlikely that females mate with several males to ensure an adequate sperm supply or to obtain nutrients. Females, in fact, appear to go out of their way to minimize the quantity of sperm and seminal fluid they accept during a single mating. The strong tendency of C. scorploides females to accept only one spermatophore from each of several males could be explained by a need to ensure that they obtain sufficient sperm, if second and subsequent spermatophores were sperm deficient. However, this is not the case. Sperm counts of rejected spermatophore packets have demonstrated that these packets do not contain fewer sperm than first spermatophore packets “stolen” from mating pairs (see Chapter 2). If the benefits associated with mating with several males primarily involved nutrient donations, then females would not be expected to minimize the number of packets accepted from a male. Moreover, there is no evidence that the number of sperm packets per se
influences female reproductive success. Females accepting two packets from a single male did not produce more nymphs than females accepting only one.

The inability of these material benefit hypotheses to explain the enhanced reproductive success of multiply-mated *C. scorpionides* females suggests that some form of genetic advantage is involved. As pointed out above, if females mate with several males to increase the likelihood of having their eggs fertilized by intrinsically superior males or sperm (Birkhead et al. 1993), male quality should exert a consistent effect on reproductive success across females. The finding that, in this pseudoscorpion, insemination by a particular male can have radically different fitness consequences for his two mates provides no support for the intrinsic male quality hypothesis. It is, however, consistent with the hypothesis that females are polyandrous because this behavior provides them with postcopulatory mechanisms for dealing with the consequences of mating with a genetically-incompatible male (Chap. 2).

Preliminary evidence indicates that intrapopulation genetic compatibility in this pseudoscorpion may be disrupted by various forms of intragenomic conflict (see Cosmides & Tooby 1981; Werren et al. 1988; Hurst 1992; Haig 1993). DNA profiling of large pedigrees has identified instances of extreme segregation distortion at two hypervariable, minisatellite loci (Zeh et al. 1994 and manuscript in preparation). In mice and *Drosophila*, meiotic drive alleles can cause embryonic death in homozygotes and reproductive success may be reduced by 50% in matings between males and females who are both heterozygotes (Lyttle 1991). Cellular endosymbionts may also be a source of incompatibility in *C.*
*scorpioides*. Laboratory rearing has detected female biases of 2:1 or greater in the broods of field-inseminated females. This bias was heritable and associated with an unusually high embryo failure rate in lines derived from the female-biased broods (unpublished data). Tetracycline treatments are currently in progress to determine whether female bias is the result of bacterial endosymbiont infection in this pseudoscorpion. In other arthropods, such female bias results from cytoplasmic genetic elements which increase their transmission by male killing or feminizing (Ebbert 1993; Hurst & Majerus 1993; Hurst 1993), thereby generating selection on nuclear genes to restore maleness (Hurst 1991; Juchault et al. 1993). Alternatively, the embryo inviability detected in *C. scorpioides* could result from *Wolbachia*-like cellular endosymbionts which generate a form of incompatibility known as cytoplasmic incompatibility (Fine 1978). In this case, the endosymbiont increases in frequency by putting uninfected females at a reproductive disadvantage. Whereas females harboring the endosymbiont are compatible with both infected and uninfected males, uninfected females produce inviable embryos if their ova are fertilized by sperm from infected males (Barr 1980). Finally, although it remains to be determined whether genomic imprinting occurs in pseudoscorpions, viviparity in *C. scorpioides* makes embryos vulnerable to the same type of maternal/paternal genome conflict that occurs in placental mammals (Haig & Westoby 1989) and angiosperms (Haig & Graham 1991). As discussed previously (Chapter 1), the extent to which each of these various agents of intragenomic conflict actually impacts on the reproductive success of
a female depends critically on both the genetic characteristics of her oocyte and those of the penetrating sperm. Intragenomic conflict thus generates variability in genetic compatibility.

If meiotic drive, cellular endosymbionts and imbalance in genomically-imprinted genes do cause incompatibility in *C. scorpioides*, polyandry could reduce the risk of brood failure by enabling females to exploit postcopulatory mechanisms (sperm competition, female choice of sperm and re-allocation of maternal investment) for minimizing the risk and/or costs of fertilization by genetically-incompatible sperm. For example, since viable sperm production may be halved in males carrying a meiotic drive allele, heterozygous females could reduce the risk of producing defective offspring by mating multiply and placing such males at a disadvantage in sperm competition (Wu 1983; Haig & Bergstrom 1995). In the case of cellular endosymbionts, polyandrous, infected females could theoretically enhance their fitness through postcopulatory choice of sperm carrying male-restoring alleles. DNA profiling suggests that this may occur in *C. scorpioides*. In one striking case, offspring of a female mated to three males exhibited all three sex ratio patterns when categorized according to which male was the father (male to female ratio: sire A: 2:3; sire B: 17:4; sire C: 0:6; \( P = 0.0002 \), Fisher exact test). Interestingly, the male achieving the highest reproductive success produced the highest ratio of sons to daughters. For viviparous organisms such as mammals and pseudoscorpions, a potentially important, additional benefit of polyandry is that mixed paternity enables females to shunt resources from abnormally-developing embryos to viable
offspring, thereby avoiding the complete brood loss which might result from mating with a single, genetically-incompatible male.

If genetic compatibility is an important criterion for female choice, then, as G.A. Parker has pointed out, "... to expect selection to produce mate choices which take account of the combined result of a female's genotype and that of her suitor is indeed to have faith." (Parker 1992). Polyandry may provide females with a partial solution to this predicament. By mating with several males, females may be relying less on faith and more on postcopulatory sexual selection to secure genetically compatible fathers for their offspring.
CHAPTER 4

HOMOZYGOITY, SELF-RECOGNITION AND AGGRESSIVE ABILITY IN THE SEA ANEMONE, ANTHOPLEURA ELEGANTISSIMA

In a recent study of aggressive behavior in the sea anemone, *Anthopleura elegantissima*, Ayre & Grosberg (1995) demonstrated the existence of a transitive dominance hierarchy, with dominance ranks of clones strongly correlated with mean number of acrorhagi (modified tentacles with concentrations of stinging cells) per polyp. The authors suggest that extensive clonal diversity and interclonal variation in agonistic behavior are maintained in the face of intense, interspecific competition for space partly as a result of trade-offs between competitive ability and other components of fitness such as sexual reproduction and asexual proliferation. The aim of this chapter is not to criticize the conclusions reached by Ayre & Grosberg (1995), but rather to point out a striking relationship in their data which may have important implications for understanding the relationship between self-recognition and aggression in marine invertebrates.

To establish that each clone was genetically distinct from all other clones used in the aggression experiments, Ayre & Grosberg (1995) screened a sample of polyps from each aggregation for 11 variable, enzyme-encoding loci. The authors used two methods, "medal play" and "match play," to derive
competitive ranks ($R_{\text{medal}}$ and $R_{\text{match}}^*$ respectively) based on replicated pairwise interactions between seven clones. My re-analysis of the data presented in the paper (Ayre & Grosberg 1995: Table 1 and Fig. 4) revealed that dominance rank was highly correlated with the number of loci at which a clone was homozygous (Fig. 4.1). Comparison of the linear regression of dominance rank on number of homozygous loci with regression of dominance rank on mean number of acrorhagi demonstrated that homozygosity accounted for a higher proportion of variation in dominance rank ($R_{\text{medal}}^*; r^2 = 0.86, P = 0.0025; R_{\text{match}}^*; r^2 = 0.69, P = 0.020$) than did mean number of acrorhagi ($R_{\text{medal}}^*; r^2 = 0.57, P = 0.050; R_{\text{match}}^*; r^2 = 0.30, P = 0.205$). When both acrorhagi and homozygosity were included as independent variables in a multiple linear regression, the partial regression coefficient ($\pm$SE) for number of homozygous loci ($\beta_{\text{Hom}}$) was significant ($R_{\text{medal}}^*; \beta_{\text{Hom}} = -1.22\pm0.41$, one-tailed $P = 0.020; R_{\text{match}}^*; \beta_{\text{Hom}} = -1.42\pm0.59$, $P = 0.037$). By contrast, the partial regression coefficient for mean number of acrorhagi ($\beta_{\text{Acr}}$) was not significant ($R_{\text{medal}}^*; \beta_{\text{Acr}} = -0.04\pm0.10$, $P = 0.357; R_{\text{match}}^*; \beta_{\text{Acr}} = 0.07\pm0.14$, $P = 0.317$).

The results of this multiple linear regression suggest that the correlation between acrorhagi and dominance may simply be a byproduct of the positive correlation between acrorhagi and multilocus homozygosity ($r = 0.76$). Indeed, this interpretation provides a parsimonious explanation for the results obtained for clone E. This clone achieved only an intermediate dominance rank but
Fig. 4.1. Linear regression of dominance rank (as assessed by the medal play procedure of Ayre and Grosberg (1995)) on the number of homozygous loci exhibited by each A. elegans (s)ima clone compared to linear regression of dominance rank on the mean number of acorhagi possessed by each of the clones.
possessed by far the greatest number of acrorhagi (26 versus a mean of 14 for the remaining six clones), with the result that the relationship between acrorhagi and dominance rank was only marginally significant (medal play) or nonsignificant (match play) unless clone E was treated as an outlier and excluded from the analysis (Ayre & Grosberg 1995). No such ad hoc treatment of the data was required to detect a significant relationship between number of homozygous loci and dominance rank.

Although, only a limited number of clones and electrophoretic loci were surveyed in this study, the strength of the correlation between homozygosity and dominance rank is nonetheless striking and warrants investigation. In many sessile, clonal invertebrates, encounters between conspecifics are characterized by passive interactions between clonemates or close kin and aggressive responses to unrelated individuals. It is often argued that aggression is triggered by allelic differences at one or few highly polymorphic "recognition" loci (Grosberg 1988) similar to the major histocompatibility complex of vertebrates. Although there is evidence for a major histocompatibility complex-like recognition system in the protochordate, Botryllus schlosseri (Scofield et al. 1982), whether other invertebrates such as Cnidaria possess such a recognition system remains to be determined. Clearly the ability to discriminate between self and non-self is a precondition for agonistic behavior yet the actual mechanisms by which invertebrates make such distinctions and how these mechanisms may limit the capacity for adaptive aggressive response have received inadequate attention in the ecological and evolutionary literature.
In a recent model based on Burnet's (1971) concept of self-recognition, Humphreys & Reinherz (1994: 320) hypothesize that, whereas in vertebrates, "the primary focus of immune receptor molecules on T cells and B cells is directed outward for specific recognition of foreign molecules," in invertebrates, "... the expression of diversity, positive selection and clonal expansion are directed inward towards self-recognition." If alloreognition involves responses at several loci, this model of invertebrate immune recognition based on active recognition of self can provide a framework for understanding how homozygosity could act to enhance competitive ability in *A. elegantissima*. In essence, homozygosity creates a pure identity: the greater the number of loci at which a clone is homozygous, the greater the number of interactions in which that clone can distinguish between self and non-self. By contrast, the possession of two distinct alleles creates a dual identity at any given locus: the greater the number of loci at which a clone is heterozygous, the greater the number of interactions in which that clone cannot distinguish between self and non-self. To illustrate the point, in interactions between clones of genotypes AA, AB and BB, the two homozygotes can recognize each other and the heterozygote as non-self. By contrast, the heterozygote recognizes neither clone AA nor clone BB as non-self. In this simplified single-locus example, by responding aggressively to the passive, heterozygote opponent, the homozygotes will dominate in competition for space.

The fact that homozygosity and aggressive ability covary continuously in *A. elegantissima* is consistent with the suggestion that numerous loci contribute in
an additive fashion to allorecognition (Koyama & Watanabe 1983; Scofield & Nagashima 1983). A multilocus recognition system could also explain why two pairs of clones, A vs. E and D vs. G, which in each case differed by a total of only one allele across the 11 enzyme-encoding loci, responded non-aggressively to one another in the study by Ayre & Grosberg (1995). Assuming such a system of recognition involving multiple loci with relatively few alleles per locus, the level of homozygosity at the electrophoretic loci assayed by Ayre & Grosberg (1995) should be positively correlated with level of homozygosity at the recognition loci. However, as theoretical analyses have shown (Chakraborty 1981; Turelli & Ginzburg 1983), such correlations may be confounded by the number of loci assayed and the reproductive and demographic characteristics of the population in question. Nonetheless, the 11 variable loci surveyed by Ayre & Grosberg (1995) compares favorably to numbers of polymorphic loci screened in investigations aimed at assessing genome-wide heterozygosity (e.g., five loci in Gaffney 1990; 11 loci in Pecon Slattery et al. 1991).

If, as Ayre & Grosberg (1995) suggest, aggressive ability determines the outcome of competition for space in A. elegantissima, selection should act to weed out all but the most aggressive clones. Nevertheless, natural populations of this sea anemone exhibit high levels of clonal diversity even on a local scale (Smith & Potts 1987). Multilocus homozygote superiority in competitive ability could explain this persistent variation. In the study by Ayre & Grosberg (1995), both of the two top-ranked clones were homozygous for 10 of the 11 variable loci screened. While this high level of homozygosity is apparently not the
outcome of inbreeding (D. J. Ayre & R. K. Grosberg, personal communication), by increasing homozygosity, inbreeding could potentially be an important factor contributing to aggressive ability in other sessile, marine invertebrates. In such a situation, competitive ability would be a non-heritable trait, dominance hierarchies would break down as a consequence of outbreeding, and clonal diversity would not be reduced by competition for space. Even if a clone's tendency to inbreed were genetically-based, homozygote superiority would not erode genetic variability, if all homozygotes, irrespective of allele type, were equally competitive, as my proposed mechanism of enhanced self-recognition implies.

The homozygote aggressive superiority hypothesis proposed here could, in principle, be tested by crossing pairs of highly homozygous, top-ranked clones, thereby creating heterozygous offspring which, unlike their parents, should exhibit low competitive ability. Unfortunately, such a test using _A. elegantissima_ is currently not feasible, since attempts to rear _Anthopleura_ species beyond the larval stages have to date been unsuccessful.
BIBLIOGRAPHY


Barlow, D.P., Stöger, R., Herrmann, B.G., Saito, K. & Schweifer, N. 1991 The
mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature, Lond. 349, 84-87.


Fine, P.E.M. 1978 On the dynamics of symbiote-dependent cytoplasmic


Haig, D. & Bergstrom, C.T. 1995 Multiple mating, sperm competition and meiotic


Ecol Evol. 9, 431-439.


Siva-Jothy, M.T., & Tsubaki, Y. 1989 Variation in copulation duration in Mnaïs pruinosa pruinosa Selys (Odonata: Calypterygidae) 1. Alternative mate


Taylor, C.E., Pareda, A.D., & Ferrari, J.A. 1987 On the correlation between


Turelli, M., & Ginzburg, L. R. 1983 Should individual fitness increase with heterozygosity? *Genetics* 104, 191-209.


Zeh, D.W. & Zeh, J.A. 1996a Female promiscuity undermines large-male mating advantage in the harlequin beetle-riding pseudoscorpion. (in prep.).


