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Sex gene pool evolution and speciation: A new paradigm

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In this paper, we review the literature on the growing body of data demonstrating the rapid evolution of sex and reproduction related (SRR) genes and show how a paradigm shift to the study of SRR genes can provide new approaches to solving some of the old problems in evolutionary biology. The argument is based on (1) the growing scope and importance of sexual selection in evolution, (2) the growing number of case studies showing rapid evolution of sexual traits in a wide variety of taxa, (3) the faster rate of DNA sequence divergence in genes affecting sexual function and fertility, (4) the evidence for the involvement of novel traits / genes in sexual functions, and (5) a proposed *sex / non-sex* dichotomy of the gene pool affecting viability *versus* fertility. It is argued that the adoption of the *sex / non-sex* dichotomy of genes / traits can provide new perspectives on such problems as species concepts, modes (allopatric/sympatric) of speciation, Haldane's rule, reinforcement, and the founder effect. It is proposed that the evolutionary study of genes affecting viability *versus* fertility is the key to understanding the genetic basis of speciation.

INTRODUCTION

Sexual reproduction constitutes the core of the Biological Species Concept (BSC) yet sex and fertility traits have received little attention relative to their importance in evolution and speciation. Darwin gave special treatment to sexual selection in order to draw attention to the intricate process of mate selection, the astonishing levels of sexual dimorphisms observed in many species (especially higher organisms including man) and to show that sexual selection is not without cost especially to the male members of the species. In contrast to natural selection, which Darwin perceived to work through changes produced in response to the varying general conditions of life, sexual selection operates through female choice and male competition for mates. Males, with their superior strength, pugnacity, armaments, unweildy passion and love songs, are almost always the more active and most often, the initiator of sexual interactions (Darwin, 1871). In spite of its importance in mate selection and reproduction, sexual selection has always been treated as somewhat of a special case that plays a secondary role in comparison to

natural selection. Sexual selection is often considered subsumed under natural selection but the latter's strong connotation with only viability and survival still persists. The lack of focus on sexual traits in evolutionary studies is unexpected given their importance in the early systems of classification, particularly in plant and insect taxonomy.

Part of the reason for this dichotomy is, of course, Darwin's own emphasis on competition for survival in his theory of natural selection. Competitive survival became synonymous with natural selection and evolution. During the evolutionary synthesis, it formally became the theoretical basis of population genetics when using models based on genes affecting viability rather than fertility became the standard approach (Lewontin, 1974; Lewontin 2000). Most theoretical models as well as experimental research were predominantly based on viability selection even though it was known that fertility rather than viability comprised the larger fraction of total fitness (Prout, 1971). The evolutionary synthesis was based on pluralistic approaches which sought generality and therefore it is not uncommon to see all possible factors and evolutionary processes mentioned in seminal publications (Dobzhansky, 1937; Huxley, 1942; Mayr, 1942). The synthesis primarily focussed on producing an evolutionary

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theory that was acceptable, general, and central to all biological systems. This does not mean that reproductive traits were not studied; it is simply that these traits were of no special interest and were studied as part of the ensemble of traits affecting overall fitness of genes and genotypes. Plants, with their wide variety of breeding systems, were a notable exception (Stebbins, 1950) and the rapid evolution of flower traits in insect pollinated higher plants became a subject of prolific study.

A renewal of interest in sexual selection with respect to human sociobiology (Cronin, 1991; Maynard Smith, 2000), the importance of genitalic traits to insect systematics (Eberhard, 1985), and the role of sexual selection in the speciation of Hawaiian *Drosophila* (Carson, 1997) pushed sexual selection to the forefront of evolutionary biology. Sexual selection and the origin of female choice (see Andersson, 1994), speciation (Lande, 1981; Kaneshiro, 1989), and antagonistic evolution (Rice, 1996) are current areas of active research. The idea of sexual selection has also been discussed in plants (Willson and Burley, 1983). Recently a new chapter was added to the ever increasing importance of sexual selection by Eberhard (1996) who showed that sexual selection does not stop with the selection of mates but continues after copulation through sperm competition (Parker, 1970) and the action of seminal fluids during fertilization (Wolfner, 1997). This represents a drastic and important extension of sexual selection theory that encompasses molecules to morphology (Civetta and Singh, 1999). Sexual selection and sexual traits are being vigorously investigated for two additional reasons. One of these is Carson's (1997) work on the Hawaiian *Drosophila* that demonstrated the central importance of sexual selection in speciation and the other is the growing body of data demonstrating that sex and reproduction related (SRR) genes evolve faster (Singh, 2000).

In this paper we review the literature on the growing body of data demonstrating the rapid evolution of SRR genes and show how a paradigm shift to the study of SRR genes can provide new approaches to solving some of the old problems in evolutionary biology.

SEX GENE PARADIGM

1. Gene pool dichotomy

A large subset of morphological, molecular and behavioural traits are subjected to the external pressures of sexual selection. These traits / genes are, by definition, either directly or indirectly involved in some aspect of reproductive function. Sex genes contrast with non-sex genes that primarily affect development and viability and that are the subject of natural selection. Carson (1985) used the same distinction in an attempt to unify speciation theory in plants and animals. Regardless of the underlying model, especially in relation to the role of

founder events (Carson, 1985; Singh, 2000), selection is a mechanism for positive evolutionary Darwinian change (Singh, 2000). This added selective pressure, acting within a dynamic and evolving mating system, works in addition to the forces of natural selection. Under this hypothesis, one would predict sex genes to evolve at a faster rate.

Over a fifteen year period, our laboratory has surveyed the genic variation of different tissues between closely related species of *Drosophila* in order to test whether gene polymorphism and divergence could be predicted by function. *Drosophila melanogaster* and three of her sibling species formed the primary experimental material. Species of this subgroup are morphologically indistinguishable but reproductively isolated from one another (Lachaise *et al.*, 1986). In fact, when any of three sibling species are crossed to each other, hybrids are produced in which females are fertile and males are sterile. Early surveys employing one-dimensional electrophoresis (Choudhary and Singh, 1987) indicated that abundant soluble proteins diverged at a faster rate between *D. melanogaster* and *D. simulans*. Using 2D electrophoresis, which enabled a much larger group of proteins to be randomly sampled from various tissues, Coulthart and Singh (1988b) also demonstrated a higher rate of divergence amongst reproductive tract proteins between sibling species of the *melanogaster* subgroup. Specifically, they revealed that approximately 20% of male reproductive tract proteins sampled in *D. melanogaster* had no detectable homologue in *D. simulans* and vice-versa (both species possess a common ancestor 2–3 million years old), the high divergence between species can be attributed mostly to loci that are not polymorphic, and expression levels of many reproductive tract proteins differ indicating a possible role of gene regulation (Coulthart and Singh, 1988a,b). A subsequent experiment confirmed that the higher rate of divergence amongst reproductive-tract polypeptides was correlated with tissue distribution and not developmental timing (i.e. larval *vs.* adult), suggesting functional divergence (Thomas and Singh, 1992).

While these results provided the basis to explain the preponderance of hybrid male sterility in *Drosophila* (Bock, 1984), another key study directed us closer to the actual process involved in the rapid divergence between closely related species. Performing a comprehensive survey of an even larger sample of proteins, tissues, and species using 2D gel electrophoresis, Civetta and Singh (1995) showed that not only were male reproductive-tract proteins more highly diverged (they also studied closely related species of the *D. virilis* clade), but proteins from the female reproductive-tract were significantly more diverged relative to non-reproductive proteins. These data support the existence of selection on traits involved in sex and reproduction in *both* sexes and unveil a previously obscure domain in evolutionary biology-the

cryptic and inscrutable nature of female choice does not render itself as much to investigation as the male.

We have further argued that reproductive-tract proteins are not the only proteins to show high divergence between species (Civetta and Singh, 1998a). Sexual traits encoded by genes not expressed in the reproductive tract may certainly be under the influence of sexual selection as well. Below, we have listed three categories of SRR genes along with relevant examples, particularly from the *Drosophila* literature.

a. Primary sexual traits: Numerous examples of highly diverged traits involved in male reproduction have been documented. For centuries, external male genitalia have been used by taxonomists in order to distinguish closely related insect species. For example, species of the *melanogaster* subgroup can only be identified (other than by interspecific crosses) by conspicuous differences in the shape of the posterior lobes of their genital arches (Lachaise *et al.*, 1986). Eberhard (1985) has shown this common theme of unique species-specific male genitalia to be found in a variety of animals. Internal sexual organs such as testes and accessory glands have likewise been shown to be highly diverged between species. In their seminal study, Patterson and Stone (1952) documented the large differences in testes size and shape between various *Drosophila* species. Using a principle component approach, Civetta and Singh (1998b) showed that reproductive characters such as testis length and area, as well as the genital arch's posterior lobe, revealed the highest proportion of variation between species of the *melanogaster* subgroup when compared to nonreproductive traits such as wing, femur and malpighian tubule length. The male gametes themselves are also quite diverged. Sperm length is considered one of the most rapidly evolving traits in insects, particularly *Drosophila* (Joly *et al.*, 1989; Karr and Pitnick, 1996; Pitnick and Markow, 1994). Joly *et al.*'s (1995) study on *D. bifurca* reveals a coiled morphology and "peashooter" strategy in storing and transferring sperm into the female, alluding to the presence of extremely diverse sperm release mechanisms in *Drosophilids*.

Recently, an increasing number of molecular studies on seminal fluid proteins have reported their rapid evolution in *Drosophila*. These molecules are transmitted from the male to the female during copulation and influence her behaviour and physiology (for review, see Wolfner, 1997). For example, females with deposited male seminal products are less responsive and less attractive to male flies and decrease their receptivity to further mating for a substantial duration of time. An assortment of accessory gland proteins (*Acps*) have been shown to be quite diverged, even among closely related species, in numerous studies (Aguadé *et al.*, 1992; Clark *et al.*, 1995; Tsaur and Wu, 1997; Cirera and Aguadé, 1997). In a series of

elegant studies, Wolfner and her colleagues have shown that these proteins are involved in fertilization using a variety of mechanisms including the "corralling" of sperm for storage (*Acp36DE*) and the stimulation of egg-laying rate (*Acp26Aa*) (Wolfner, 1997).

Similar studies have shown that proteins involved in the development and maturation of sperm itself have also been evolving rapidly in *Drosophila*. In the search for a hybrid male sterility gene, Ting *et al.* (1998) cloned the male sterility gene, *Odysseus* (*Ods*), and discovered it to include a highly diverged homeodomain. Amongst sibling species of the *melanogaster* subgroup, the number of replacement substitutions in this domain far exceed the number of silent substitutions. Interestingly, between *D. simulans* and *D. mauritiana* (common ancestor less than one million years ago), *Ods* has been shown to exhibit the same degree of divergence as *Drosophila* and man (which have diverged approximately 700 million years ago). In another study, the testes-specific gene, proteosome *alpha4t2*, revealed low identity (59%) between *D. melanogaster* and *D. virilis* relative to the somatically expressed proteosome *alpha4* which shared 90% sequence identity between the same two species (Beloté *et al.* 1998). Mammalian evolution has also given us an example of a highly evolved sperm protein. Amongst hominids and Old World monkeys, the exons in protamine P1, a histone substitute which binds sperm DNA during spermatogenesis, evolve more rapidly than introns, indicative of positive selection (Rooney and Zhang, 1999).

Although the data of Civetta and Singh (1995) suggests a higher rate of divergence in both male and female reproductive genes, specific examples of highly evolving female reproductive traits/proteins are lacking. It was only recently that a handful of studies have revealed faster divergence of female reproductive traits but such examples are usually associated with male reproductive function (Clark *et al.*, 1995; Rice, 1996; Simmons and Siva-Jothy, 1998). In one such investigation, Pitnick *et al.* (1999) demonstrated the rapid divergence of female reproductive tract morphology as a correlate to sperm morphology. We encourage more research to be performed on female reproductive characters as this will prove to be effective in discovering novel rapidly evolving traits, and may enable us to understand which genes are under strong selection.

b. Sex determination genes: A second class of sex genes consists of the loci that determine sex. Genes involved in this pathway are primarily responsible for the determination of sexual dimorphism found in most organisms. In *Drosophila*, differences between sexes include primary reproductive organs as well as secondary sexual characteristics such as pheromonal profile, presence/absence of sex combs and behavioural differences in mating. Although conservation of function and structure of sex determination pathways would be expected because

of the important nature of these differences, sex determination pathways are among the most rapidly evolving of developmental systems (Hodgkin, 1990). For example, sex in *Musca domestica* is determined by major loci while in the closely related genera, *Drosophila*, sex is determined by the ratio of autosomes to X-chromosomes. Even different strains from the same species of *M. domestica* have been shown to possess divergent mechanisms ranging from male heterogamy to female heterogamy to maternal effects (Dubendorfer, 2000).

In addition to this particular system's plasticity, rapid sequence divergence is observed in genes of the sex-determination pathway, even between closely related species. *Transformer*, one of the key sex determining genes in *Drosophila*, has been shown to be one of the most diverged proteins between *D. melanogaster* and *D. simulans* (O'Neil and Beloté, 1992; Kulathinal *et al.*, 2000). *Transformer-2*, which acts in concert with *transformer*, also is highly diverged between species of the *melanogaster* subgroup (R.J.K., unpublished data). Rapid sequence divergence is similarly observed in sex-determining genes from other organisms that employ quite different mechanisms. Much of the *Sry* locus of mammals has been demonstrated to be rapidly diverging (Hawkins, 1994). Similarly, the *transformer-1* and *transformer-2* loci found in nematodes have also been shown to be among the fastest evolving sequences known in worms (Kuwabara, 1996).

c. Behavioural / mating traits: Traits that are involved in a wide range of mating cues and preferences also fall under the generalized category of sex traits. Such characters, often species- or genus-specific, can be quite divergent between species. The Hawaiian *Drosophilids* are a primary example (for review, see Carson, 1997). Although they share a common ancestor less than five million years ago, many of these species possess differences that seem to exaggerate certain elements of mating such as elongated antennae, forelegs, and wing patterning (Kaneshiro and Boake, 1997). Even within species, certain populations are behaviourally isolated from one another. Wu *et al.* (1995) showed that populations of *D. melanogaster* originating from Zimbabwe are strongly sexually isolated from other global populations through mating barriers.

The complex phenotype of *Drosophila* courtship involves many genes (Hall, 1994) and certain genes seem to play a larger role than others depending on the particular species groups. For example, cuticular hydrocarbons, which have been implicated as pheromones, show differential expression patterns between species of the *melanogaster* subgroup (Ferveur and Jallon, 1993; Coyne, 1996). In *D. simulans* and *D. mauritiana*, the hydrocarbon, *cis* 7-tricosene, is found in high amounts in both males and females while it is only found in males in *D. melanogaster* and *D. sechellia* (Coyne, 1996). Differing amounts of this

compound have previously been shown to effect wing vibration involved in courtship song. Male courtship song itself has also been shown to be highly species-specific (Bennet-Clark and Ewing, 1970). The *period* gene regulates circadian rhythms such as male courtship song cycles (Yu *et al.*, 1987) and a number of studies have revealed a large amount of sequence divergence (Thackeray and Kyriacou, 1990; Kliman and Hey, 1993). An interesting feature of *period*'s divergence is the region surrounding and including a threonine-glycine repeat that has been implicated in determining the species-specific differences in *Drosophila* lovesong cycle (Wheeler *et al.*, 1991).

2. Evidence for faster evolution of sex genes

In an attempt to summarize much of the anecdotal evidence propounding a faster rate of divergence among sex genes, Civetta and Singh (1998a) compared the degree of sequence divergence between a number of *Drosophila* species. Genes were classified according to their function - those that were involved in any aspect of sex and reproduction (i.e. mating, gametogenesis or fertilization) were grouped as sex genes and the remaining were grouped as non-sex genes. The ratio of Kr (nonsynonymous substitutions per nonsynonymous site) to Ks (synonymous substitutions per synonymous site) was found to be significantly higher among the sex gene subset between the closely related species pair, *D. melanogaster* and *D. simulans* (N=17 loci) as well as the more distant species pair, *D. melanogaster* and *D. pseudoobscura* (N=22 loci). (This study also revealed higher sex-gene divergence between two species of nematodes, *Caenorhabditis elegans* and *C. briggsae*.)

Using the same classification as Civetta and Singh (1998a), we have updated the divergence data between *D. melanogaster* and *D. simulans* to include 56 orthologous loci (Fig. 1). These sequences and their summary statistics can be found in Kulathinal *et al.* (2000). (We excluded the 3 *anon* genes since their functions remain unknown at this time.) Measures of divergence, Kr and Ks, and their ratio, Kr / Ks, were all found to be significantly larger in sex genes compared to non-sex genes (Kr, $t=3.42$, $P=0.012$; Ks, $t=2.27$, $P=0.027$; Kr/Ks, $t=3.39$, $P=0.001$). Even after excluding the rapidly evolving seminal fluid gene, *Acp26Aa*, all three divergence values remained significantly larger in sex genes. It is interesting to note that synonymous substitutions per site, Ks, is also significantly higher in sex genes. A highly significant positive correlation has been previously reported between Ks and Kr and a number of explanations including differential mutation rate, codon bias and doublet mutations, have been offered (see Smith and Hurst, 1999).

The higher divergence of sex genes on average may present a general pattern among organisms. Table 1 shows examples of rapidly evolving sex genes from a wide assortment of species. In a survey of 15 homologous loci

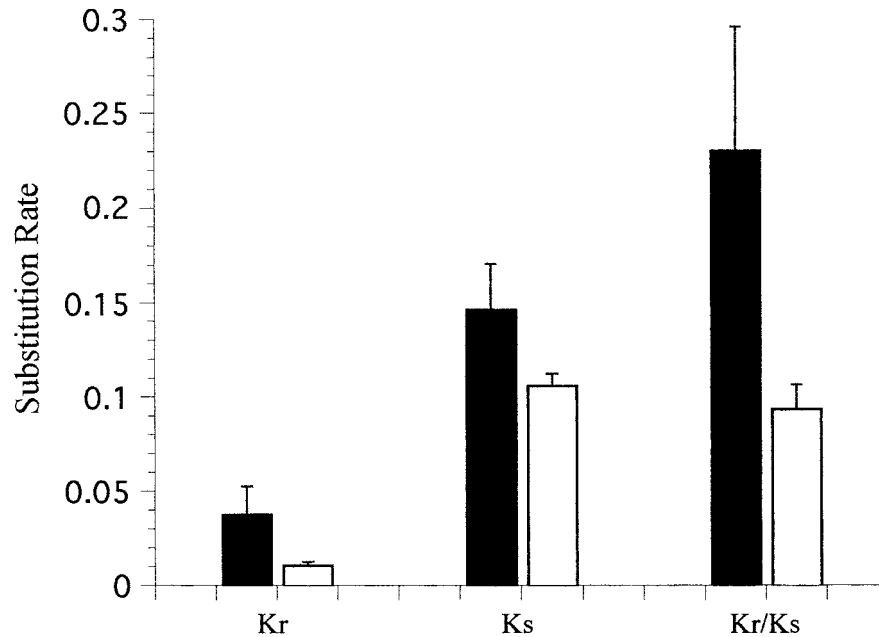


Fig. 1. Divergence rates of sex vs. non-sex genes between *D. melanogaster* and *D. simulans*. Available sequences, common to both species, were categorized into sex related ($N = 10$) and non-sex related ($N = 46$) gene groups according to the criteria described in Civetta and Singh (1998). Sex genes are plotted as solid bars and non-sex genes are plotted as open bars. Kr, nonsynonymous substitutions per nonsynonymous site, Ks, synonymous substitutions per synonymous site, as well as the ratio of Kr : Ks are compared between both classes of genes. Standard error bars are indicated.

Table 1. Examples of high sex-gene divergence in different taxa

Taxa	Gene(s)	Classification	Divergence	Ref.
Fungi	<i>STE2</i> , <i>pra1</i>	Mate recognition (pheromonal)	-both proteins show low identity	1, 2
Algae	<i>fus1</i> <i>mid</i>	Gamete Recognition Sex determination	-both genes show low codon bias and identity between <i>C. reinhardtii</i> species	3, 4
Plants	<i>S-locus</i>	Gamete Recognition	-high Kr and heterogeneous distribution	5, 6
Invertebrates				
Marine	<i>lysin</i> <i>lysin</i> receptor	Fertilization (sperm) Fertilization (egg)	-higher Kr than Ks between species -variation in receptor parallels <i>lysin</i>	7 8
	<i>bindin</i>	Gamete Recognition	-high Kr:Ks ratio between species	9
Terrestrial	<i>tra-1</i> , <i>tra-2</i>	Sex determination	-lowest protein identity (out of 15 loci) between <i>C. elegans</i> & <i>C. briggasae</i>	10, 11
Mammals	<i>Pem</i> <i>Sry</i> <i>protamine</i>	Gametogenesis Sex determination Spermatogenesis	-highest diverged homeodomain rat/mice -higher Kr than expected among primates -higher Kr than expected among primates	12 13, 14 15

(1) Marsh and Herskowitz 1988 (2) Bakkeren and Kronstad 1994 (3) Ferris *et al.* 1996 (4) Ferris and Goodenough 1997 (5) Clark and Kao 1991 (6) Uyenoyama 1997 (7) Lee *et al.* 1995 (8) Swanson and Vacquier 1998 (9) Metz and Palumbi 1996 (10) deBono and Hodgkin 1996 (11) Kuwabara and Hodgkin 1996 (12) Sutton and Wilkinson 1998 (13) Tucker and Lundrigan 1993 (14) Whitfield *et al.* 1993 (15) Rooney and Zhang 1999

between *C. elegans* and *C. briggasae*, the sex-determination loci, *tra-1* and *tra-2*, possessed the lowest identity (Kuwabara and Hodgkin, 1996). Amongst mammals, genes that are involved in sex and reproduction have recently been also shown to have higher than average rates of divergence. Wyckoff *et al.* (2000) found a higher re-

placement substitution rate between humans and Old World monkeys among male reproduction-associated genes but noted the possibility of a nonrandom bias in the selection of these genes. A recent large-scale survey of mammalian orthologs has again directed our attention to a higher than average rate of divergence in both male

and female reproductive tracts. Duret and Mouchiroud (2000) arranged 2400 human-rodent orthologs to different tissues by comparing each ortholog to 19 available tissue-specific EST libraries. After calculating divergences, it was shown that sequences expressed in the testis (N=100) and uterus (N=59) had a significantly larger replacement substitution rate than sequences expressed in the least diverged tissue, the brain ($p < 0.005$), and that the rates of nonsynonymous divergence of testis- and uterus-expressed proteins were not significantly different than sequences expressed in the most diverged tissue, the liver. These findings are remarkably similar to those reported from our lab from 2D protein studies amongst the sibling species of *Drosophila melanogaster* (Thomas and Singh, 1992).

FURTHER DICHOTOMY OF SEXUAL TRAITS

An obvious *caveat* to this dichotomous classification is that the partitioning of genes and traits into mutually exclusive groups, as performed here, is bound to be imperfect. This is due to genes exhibiting pleiotropic effects that may affect both sexual and nonsexual functions. Still, it would be hard to deny that with respect to certain well defined functions such as sex and reproduction, not all genes and proteins are equally important. We have used a general framework (Singh, 2000) to propose that there are two components of the gene pool: (1) a *sex gene pool* which effects the development and differentiation of the 'sexual machinery' involved in mating and reproduction, and (2) a *non-sex gene pool* which affects physiological, morphological and behavioural traits involved in survival and adaptation (Table 2). Viability traits affecting development, differentiation and survival behaviour are expected to have, with one exception, only indirect, pleiotropic effects on reproductive isolation. The exception applies to morphological traits such the tail length and crest morphology in birds, or newly evolved traits such as male sex combs in *Drosophila planitibia* (Carson, 1997)

which take the role of secondary sexual traits. These traits can have strong and direct effects on reproduction and reproductive isolation. In general, this dichotomy of traits and gene pools into *sex* and *non-sex*, can serve as a new approach to the genetic studies of speciation. It is expected that different taxa, depending on their life histories and reproductive behaviour, will differ in the roles of sex and non-sex genes, and in the former case, in the development of reproductive isolation and speciation.

Reproductive traits can be further partitioned into *pre mating* and *post mating* which have different expectations regarding levels of divergence and roles in different theories of speciation. A series of dichotomies can follow. Postmating traits may be of two types, *prezygotic* and *postzygotic*, and this distinction is important in distinguishing such divergent processes, with completely different etiologies, as sperm competition and hybrid sterility. In the latter case, *postmeiotic* defects (e.g., mutations affecting sperm individualization and motility) have been shown to accumulate faster than *premeiotic* defects (Kulathinal and Singh, 1998).

One of the strongest arguments in favour of the trait dichotomy presented in Table 2 comes from the nature of reproductive defects observed in species hybrids. It is very often the case that hybrids between closely related species are physically fit (and often superior to parents) but sterile (Bock, 1984). In other words, the sexual machinery typically exhibit the first sign of species breakdown. In cases where hybrids are fully viable and fertile, traits affecting mating behaviour such as courtship song and pheromones, are often found affected. Another argument in favour of the sex/non-sex trait dichotomy is the observation that in *Drosophila*, viability mutations tend to affect both sexes equally but sterility mutations tend to be sex-specific (Lindsley and Lifschytz, 1972). Genes affecting fewer tissues or genetic systems are likely to diverge more than ubiquitously expressed genes (Coulthart and Singh, 1988b).

Table 2. Sexual vs. non-sexual traits: Relative effects on components of fitness and relevance to theories of speciation

Functional Grouping of Traits	Effects on Fitness		Relevance to Theories of Speciation
	Viability	Reproduction	
Non-sexual Traits: Development, metabolism, physiology	DIRECT & STRONG	Indirect & Weak	¹ Allopatric Speciation, ² Sympatric Speciation
Primary Sexual Traits: Mating behaviour, gametogenesis, fertilization,	Indirect & Weak	DIRECT & STRONG	³ Organizational Theory, ⁴ Mate Recognition ⁵ Genetic Transilience
Secondary Sexual Traits: Morphology (traits adapted to sexual function)	DIRECT & STRONG	Indirect & STRONG	⁶ Runaway Selection, ⁷ Reinforcement

¹Mayr (1940); ²Bush (1969); ³Carson (1982); ⁴Paterson (1985); ⁵Templeton (1980); ⁶Lande (1981); ⁷Dobzhansky (1951)

NEW SYNTHESIS

Resolution of problematics in speciation

The functional classification of the gene pool into sex and non-sex related components adds renewed insight to our understanding of speciation. First, it directs our attention to a subset of loci that may be preferentially involved in the early stages of species formation. As a general class, sex and reproduction related genes have been shown to participate in the differentiation of closely related species amongst a wide range of taxa, thus providing a universality to the species problem (Singh, 2000). Second, this classification may serve to synthesize many contentious issues in speciation. By incorporating the sex gene paradigm and all its various dichotomies (as listed above), much needed clarity will be gained in such debated topics as species concepts and specific modes of speciation.

a. Species concepts: Over the last century, the species concept has evolved from a typological definition to an operational one (Mayr, 1963). This progression has, to a greater extent, shifted our primary focus from explaining patterns of diversity to understanding the various processes involved in early species formation. Of course, much of the credit belongs to both Dobzhansky (1937) and Mayr (1963) who developed the Biological Species Concept in order to define species limits. According to the BSC, gene flow, which acts to homogenize populations, can be “prevented in nature by a reproductive isolating mechanism or by a combination of such mechanisms” (Dobzhansky, 1970). While the BSC and other isolation concepts emphasize the exclusiveness between species, other species concepts underscore the inclusiveness within species. Paterson (1985), for example, argues that species are a common “field of recombination” which share common fertilization systems. Carson (1997) considers isolation to be merely incidental outcomes of earlier divergence driven by forces such as sexual selection (Carson, 1985). Templeton’s (1989) cohesion concept also solicits the inclusive nature of species. A detailed discussion can be found in Singh (2000).

Utilizing the concept of the sex gene pool, the commonality of *reproduction* can be used to connect the various species concepts. In this paradigm, sex-related genes preferentially change in early species formation. Isolation concepts which effectively preclude gene flow between species, and recognition or cohesive concepts which allow for the evolution of fertilization systems within species, both utilize the sex gene pool in order to generate and maintain new species. Whether they employ postmating mechanisms such as hybrid sterility or mating behaviour such as mate discrimination is tax-specific. Two meta-analyses from two separate groups of *Drosophila* lend support for using reproduction-related

genes as the common thread of speciation. Coyne and Orr (1989, 1997) used genetic distances and hybridization data from over two hundred species crosses to infer that the earliest stages of postmating isolation involved hybrid male sterility. In many species of Hawaiian *Drosophila*, Carson (1997) demonstrated that sexual traits, ranging from secondary sexual morphology to mating behaviour, have rapidly evolved within populations. The demonstrated traits varied from secondary sexual morphology to mating behaviour.

b. Sympatric speciation: Ever since Thoday and Gibson (1962) showed that disruptive selection leads to reproductive isolation, and Maynard Smith (1966) presented its theoretical possibility, the frequent occurrence of sympatric speciation in nature has been hotly debated (Bush, 1969). However, not many clear-cut cases have been demonstrated in nature. Perhaps the best example involves the threespine stickleback which has recently become a model for evolutionary biologists to study isolation in sympatry (Schluter, 1996). Three independent invasions of freshwater lakes have produced almost identical dimorphisms (in the form of benthic and limnetic ecomorphs) in sympatry. After showing the populations of different ecomorphs from different lakes are reproductively isolated but populations of the same ecomorph from different lakes are not, they conclude that “parallel evolution” has occurred through similar adaptations to similar environments.

Assuming a monophyletic origin of ecomorphs in each lake, the rapid evolution of sexual preference genes can account for their divergence. Since body size is the primary basis for mate choice and mating is assortative in sticklebacks, it is possible that individuals are simply sorting themselves out into two available and distinct habitats as a result of their mating preference (which can be assumed to be the same in all lakes). Mating system could be the initial driving force of speciation in these fishes and body size difference could simply be the inevitable consequence of assortative mating and not natural selection. In all such cases where the key trait(s) under divergent selection and mating system are the same, it is necessary to separate the role of mating from natural selection as the principle factor in speciation. Sex genes and their primary driving force, sexual selection, may play a large role in the isolation of populations in sympatry (Carson, 1985; Civetta and Singh, 1998a).

c. Reinforcement: Another contentious area in the study of speciation is the reinforcement of mating isolation between two populations after secondary contact (Dobzhansky, 1951). Two components of the sex gene pool are utilized in this model. During the initial phase of reinforcement, both populations are separated in space and time. This isolation allows for the accumulation of

postzygotic mechanisms (usually in the form of hybrid sterility). Upon secondary contact, prezygotic mechanisms (usually in the form of mating discrimination) rapidly evolve between the populations in order to decrease the number of less fit hybrids. Most models (Liou and Price, 1994; Kelly and Noor, 1996) require highly reduced hybrid fitness for reinforcement to occur. An experimental simulation of this process indeed revealed that even intermediate levels of hybrid fitness did not allow reinforcement to proceed (Hostert, 1997). Kulathinal and Singh (2000) distinguished between two models of reinforcement on the basis of gene flow. The classical model I, which is accredited to Dobzhansky, allows for partial initial isolation, interspecific hybridization, genic introgression and selective divergence. Model II requires a high degree of initial postzygotic isolation in order for the reinforcement of mating mechanisms to occur without any gene flow. In the latter model, the evolution of hybrid incompatibilities becomes the driving force in speciation while mating isolation is incidental. The reinforcement of mating isolation may result from direct selection against hybridization, or from indirect effects of ecological divergence and displacement. Accordingly, an understanding of the processes that disable hybrid reproductive function will produce a clearer picture of the forces involved in early stages of species formation. On the other hand, reinforcement with gene flow, requires the primary establishment and maintenance of substantial mating discrimination between the two populations. The relative importance of post *vs.* premating mechanisms in the reinforcement model of speciation remains its principle challenge.

d. Founder effect models: The action of drift and selection on small populations has attracted much attention in a number of speciation models such as peripatric speciation (Mayr, 1963), founder-flush (Carson, 1968) and genetic-transilience (Templeton, 1980). However, for the most part, the evolution of fertilization systems have been a missing ingredient in such models. This factor may prove to be an important influence in the speciation of small populations. Kaneshiro (1989) points out that the nature of sexual selection drastically changes when the number of potential mating partners is considerably reduced. Females may become less discriminating and eventually, as the population begins to grow, novel sexual traits may become preferred and actively selected.

In the laboratory, a number of experiments have demonstrated the evolution of premating isolation from small founding populations. However, in many of these studies, hybrid populations and multiple bottlenecks, which may represent “unnatural” or even “unrealistic” conditions, were utilized (Rundle *et al.*, 1998). In a recent attempt to test founder-effect speciation, Rundle *et al.* (1998) used laboratory strains of *D. melanogaster* and demon-

strated that under more controlled conditions (i.e. use of proper strains, single bottlenecks, replicates), isolation could not be detected. In his criticism of this study, Templeton (1999) alludes to the sensitivity of mating systems to inbreeding. We also contend that the mating system component has been missing in all tests of founder-effect. We suggest that mating systems be given the opportunity to develop within each tested line. Consequently, a bottleneck (or series of bottlenecks) will disrupt linkage disequilibria caused by sexual selection. As the population expands in its slightly different genetic and environmental context, different preferences for different traits may develop after the initial effects of genetic drift. Therefore, it is important that tests of founder effect include in their design selection pressure on SRR traits that are involved in sexual selection.

e. Haldane’s Rule: The genetics of postzygotic isolation has enormously influenced speciation theory. Much of this research has focused on Haldane’s Rule, the empirical observation that F1 hybrids preferentially show sterility or inviability in the heterogametic sex (Haldane 1922). Currently, there are two competing hypothesis that explain Haldane’s Rule. Dominance theory (Turelli and Orr, 1995), an extension of Muller’s (1940) X:A imbalance theory, uses the fact that while interactions between X-linked and autosomal factors may cause incompatibilities in males, females possess twice the number of these incompatibilities. Therefore, Haldane’s Rule would only result if the X-linked loci are recessive ($d < 0.5$). On the other hand, given that there is much more male sterility than expected, the faster-male hypothesis explains Haldane’s Rule through selection on male traits by forces such as sexual selection (Wu and Davis, 1993). The drawback to this hypothesis is that it can operate only in taxa with male heterogamy.

Faster sex gene evolution can also be used to explain Haldane’s Rule. Since genes involved in sex and reproduction diverge, on average, at a higher rate than non-SRR genes, faster-male evolution becomes a special case of faster SRR gene evolution while dominance simply becomes a promoting factor whereby in a hybrid background, recessively acting gametogenic loci are preferentially involved in hybrid incompatibilities. Not only will faster SRR evolution explain Haldane’s Rule in taxa with either male or female heterogamy, but it will serve to explain why sterility is more prevalent than inviability in hybrids amongst most surveyed taxa (Kulathinal & Singh, in preparation).

f. Role of novel sex and reproductive related genes: New tools in molecular biology such as DNA microarrays and the recent sequencing of many genomes including *Drosophila* will introduce an unprecedented influx of information to evolutionary biology. The use of these tools

will certainly transform our current notions about species differences. Sexual selection has the potential to generate genetic variation and lead to the evolution of novel traits between closely related species. Carson (1997) has observed a number of *de novo* sexual characters found between closely related species of Hawaiian *Drosophila*. In members of the *planitibia* subgroup, the distribution of foreleg cilia which are used in copulation have diverged between species, possibly due to sexual selection. *Sdic*, a novel gene in *D. melanogaster* has been found to be a fusion between two neighbouring loci and functions specifically in the testes (Numinsky *et al.*, 1998). After isolating *Odysseus*, which is involved in hybrid male sterility between *D. simulans* and *D. mauritiana*, Ting *et al.* (1998) showed the rapid divergence of its homeodomain, possibly associated with novel function in the testes. In a screen for rapidly evolving genes, Schmid and Tautz (1997) observed that a number of cDNAs do not cross-hybridize between closely related species. It will be of interest to understand the function of these genes. Another intriguing avenue of inquiry is whether genes expressed in the testes may represent a unique assortment of proteins. A recent screen of over 1600 non-redundant testis EST's has shown that approximately 6% of genes expressed in the testes have at least a two-three fold higher expression level in the testes compared to nongonadal tissues (Brian Oliver, personal communication). Hence, reproductive tissues may be a site for genes of novel function to appear and processes such as sexual selection may be the catalyst for their occurrence.

CONCLUSIONS

Contrary to general perception, the process of speciation is not like the "partitioning of the Red Sea". Speciation involves the separation of gene pools, not of individuals nor populations. Therefore, whether speciation occurs through the modes of allopatry, parapatry, or sympatry, and whether or not it involves founder effects, the partitioning of gene pools has meaning only in light of relevant traits and their functions. Do sexual and non-sexual traits show similar levels of genetic variation? Do their rates of divergence vary between species? Do sex genes show faster rates of divergence? These are some of the important questions which need to be answered before we can understand the genetic basis of speciation.

Another limiting aspect of sexual selection has been that attention is drawn only when it leads to sexual dimorphisms. Otherwise, it is ignored or the question of sexual selection does not arise. However, whether a given taxa displays sexual dimorphism or not, the two sexes are continually engaged in sexual reproduction and go through bouts of competition and cooperation generation after generation. Furthermore, many sexual dimorphic characters that are invisible to the researcher may be

perfectly conspicuous to the sexual partners. Any multi-component, coupled genetic system (in our case, male-female interactions) will respond to changes effected by each component as a result of both directed and undirected evolutionary forces. If most individuals of a population spend their time in an environment to which they are fairly adapted, the total amount of natural selection per generation, through viability differences, is likely to be less than the amount of sexual selection experienced by individuals in the same population. But unless secondary sexual traits become involved and begin to advertise, sexual selection would go unnoticed. On the time scale of generations, sexual selection may be a more important force of evolutionary change than natural selection. It is obvious that except in the case of sexual dimorphism, sexual selection may not produce directional change and yet the cycle of hidden change in the male and female sex gene pool will continue from generation to generation.

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