Reproductive mode and speciation: the viviparity-driven conflict hypothesis

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Summary

In birds and frogs, species pairs retain the capacity to produce viable hybrids for tens of millions of years, an order of magnitude longer than mammals. What accounts for these differences in relative rates of pre- and postzygotic isolation? We propose that reproductive mode is a critically important but previously overlooked factor in the speciation process. Viviparity creates a post-fertilization arena for genomic conflicts absent in egg-laying species. With viviparity, conflict can arise between: mothers and embryos; sibling embryos in the womb, and maternal and paternal genomes within individual embryos. Such intra- and intergenomic conflicts result in perpetual antagonistic coevolution, thereby accelerating interpopulation postzygotic isolation. In addition, by generating intrapopulation genetic incompatibility, viviparity-driven conflict favors polyandry and limits the potential for precopulatory divergence. Mammalian diversification is characterized by rapid evolution of incompatible feto-maternal interactions, asymmetrical postzygotic isolation, disproportionate effects of genomically-imprinted genes, and "F_2 hybrid enhancement." The viviparity-driven conflict hypothesis provides a parsimonious explanation for these patterns in mammalian evolution. ***BioEssays*** **22:938–946, 2000.**

Introduction

How new species arise during evolution is one of the most fundamental but highly contentious issues in biology. Speciation occurs when populations diverge sufficiently to become reproductively isolated; but is this isolation initiated by pre-fertilization barriers to gene flow or by postzygotic genetic incompatibility? There is currently little consensus regarding the answer to this question, and much debate over the relative importance of mutation accumulation, sexual selection, selfish genetic elements and intergenomic conflict in the speciation process.[1–6] With patterns in nature (see below) exhibiting profound between-lineage differences in the relative rates at which pre- and postzygotic isolation evolve,[7–10] a unifying theory of speciation has remained elusive. Here, we present a new hypothesis to account for the extreme disparity that exists between lineages in patterns of speciation. This *viviparity-driven conflict hypothesis* proposes that the reproductive stage at which divergence occurs most rapidly between populations is strongly influenced by the degree to which embryonic development involves physiological interactions between mother and embryo. After briefly reviewing between-lineage differences in patterns of speciation, we develop the hypothesis that postzygotic isolation should evolve more rapidly in viviparous animals than in oviparous species, because development of the embryo within the mother creates a physiological arena for genomic conflicts absent in species that lay eggs.

Determining how reproductive isolation takes place, that is, whether through mate choice and/or gametic barriers that prevent fertilization or through the postzygotic mechanisms of hybrid sterility or inviability, is fundamental to understanding speciation.[11–13] As Coyne and Orr[12] point out, "we would like to know which type of isolation (pre- or postzygotic) is most important in reducing gene flow between incipient species, for this factor would be the primary component of speciation."

Speciation patterns in nature

By far the most comprehensive data set on speciation patterns is derived from comparative studies of *Drosophila* in which pre- and postzygotic divergence were found to occur at similar rates.[12] However, in other lineages, this pattern may not apply. For example, in birds, closely-related species often differ most markedly in courtship behavior or in male song and/or breeding plumage.[14] A similar pattern appears to underlie the explosive diversification of African cichlids.[15] By contrast, in mammals, postzygotic incompatibility often occurs without appreciable divergence in sexually-selected traits.[16] Indeed, molecular assays[7–10] strongly suggest that the rate at which postzygotic incompatibility evolves differs dramatically between lineages. As Fig. 1 illustrates, bird and frog species pairs retain the capacity to produce viable hybrid offspring for up to 60 million years, an order of

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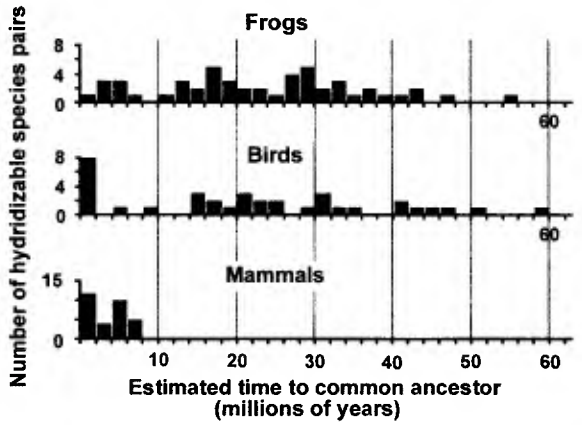

Figure 1. Hybridizability and divergence time in vertebrate lineages (compiled from Refs. 8–10). The estimated divergence times between pairs of species capable of producing viable hybrid offspring in frogs (N = 50 pairs), birds (N = 36 pairs) and mammals (N = 31 pairs) are based on an albumin clock calibrated at 1.7 immunological distance units per million years in mammals and frogs, and 0.6 immunological distance units per million years in birds. It should be noted that divergence times were determined only for species known to produce viable hybrids (either in nature or from animal husbandry studies) and thus do not represent a random sample of the taxa. While the precise form of each histogram depends, of course, on the particular species sampled, the magnitude of the disparity between mammals and the oviparous tetrapods makes it extremely unlikely that differences are due to sampling artifacts.

magnitude longer than mammals. It thus seems evident that speciation occurring through reproductive isolation at the postzygotic stage has been far less important in the oviparous vertebrate lineages than it has been in mammals.

The viviparity-driven conflict hypothesis

There is currently no generally accepted theory to account for the striking disparities between birds, frogs and mammals in patterns of postzygotic divergence. We suggest that the key to understanding these very different speciation patterns lies in considering the impact of reproductive mode on the potential for genomic conflict-driven divergence and postzygotic genetic incompatibility. According to this *viviparity-driven conflict hypothesis*, the reproductive stage at which divergence occurs most rapidly is strongly influenced by whether a species is oviparous or viviparous. In viviparous species, development of the embryo within the mother creates a post-fertilization arena for forms of genomic conflict absent in species that lay eggs.[17,18] With viviparity, conflict can arise between mothers and developing embryos, between sibling embryos within the womb and between maternal and paternal genomes within individual embryos.[19,20] Viviparity can provide a direct conduit for manipulation of the mother's physiological system by genes expressed in the embryo. The evolutionary interests of embryonic genes are likely to conflict with genes in the mother, particularly in the case of genes unique to the Y-chromosome[21,22] and paternally inherited, genomically-imprinted alleles.[23,24] The ability of viviparous females to reallocate maternal resources from defective to viable embryos can also increase the potential for genomic conflict.[17,18] Theory suggests that such reallocation from inviable male embryos to female sibling embryos exerts an important influence on the ability of cytoplasmic male killers to increase their frequency in host populations.[25] In addition, overproduction of zygotes followed by shunting of maternal resources from genetically-defective to viable embryos ("reproductive compensation") has been argued to be critical for the spread of segregation distorter alleles with lethal or semi-lethal homozygous phenotypes (e.g., mouse *t*-haplotypes, see Ref. 26).

Viviparity-driven conflicts are predicted to be most intense in polyandrous species. Multiple paternity generates relatedness asymmetries, and should favor the evolution of an aggressive paternal genome in the embryo.[19,24,27–30] The resulting tug-of-war conflict between embryonic maternal and paternal alleles over resource transfer from mother to embryo is hypothesized to be the driving force behind the evolution of genomic imprinting.[19,23] Known definitively from only marsupials,[31] eutherian mammals[32] and angiosperms,[33] genomic imprinting is a form of non-Mendelian, autosomal inheritance in which methylation and expression of an allele depend on whether it is inherited through sperm or egg. Recent single-locus models suggest that imprinting could evolve without multiple paternity.[20] However, this finding has been challenged,[30] and the weight of theoretical evidence indicates that multiple-paternity-driven conflict is essential to the evolution of imprinting.[19,27,29,30]

If the maternal/paternal gene conflict hypothesis is correct, the components of this interplay should be constantly evolving, due to a coevolutionary arms race over maternal resource allocation (see Fig. 2, left). If stimulation of resource transfer by the paternal genome and suppression by the maternal genome are viewed as polygenic traits,[27] it seems likely that maternal and paternal genomes will vary in the extent of their genetic compatibility.[17] For example, a paternal multilocus genotype of high resource transfer activity will result in normal offspring production only when paired with maternal genotypes of similarly vigorous resource transfer suppression activity. Matings between males and females at the opposite ends of their respective distributions of gene expression are likely to result either in over-demanding progeny[24] or in embryos unable to sequester adequate maternal resources (Fig. 2, right). This model is consistent with imprinting polymorphism at the human *IGF2R* locus[34] and variability in imprinting between mouse strains.[35]

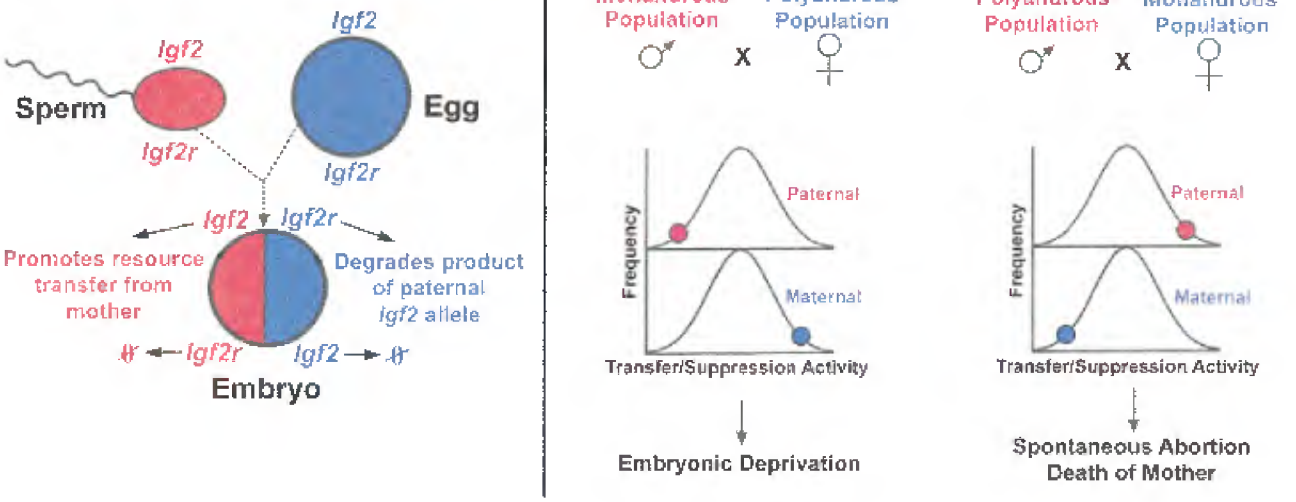

Figure 2. Mating system, divergence in genomically imprinted genes, and feto-maternal incompatibility. *Left*, *Igf2* (insulin-like growth factor type 2) and *Igf2r* (insulin-like growth factor type 2 receptor) are reciprocally imprinted genes in eutherian mammals. In the developing embryo, gene expression occurs only from the paternally-inherited (red) copy of the *Igf2* locus and only from the maternally-inherited (blue) copy of the *Igf2r* locus. The two loci function in opposition: insulin-like growth factor type 2 promotes resource transfer from the mother to the embryo but is degraded by the product of the *Igf2r*locus. With a number of loci contributing to the stimulation of resource transfer by the paternal genome and suppression by the maternal genome, maternal and paternal genomes are likely to vary in the extent to which they are genetically compatible (right). Since multiple paternity drives the evolution of more aggressive paternal genomes, such incompatibility is predicted to be greatest in crosses between populations which differ in level of polyandry.

We propose that the viviparous mode of reproduction has two major consequences for speciation. First, viviparity-driven conflict should generate perpetual antagonistic coevolution, *sensu* Rice,[5] between genes expressed in embryonic development and genes involved in maternal reproductive physiology, thereby greatly accelerating the rate at which postzygotic isolation evolves between populations. In particular, interactions between the embryo and the maternal uterine environment should diverge rapidly between populations and frequently represent the primary postzygotic barrier to hybridization.

Second, by generating genetic incompatibility within populations, viviparity-driven conflict is likely to favor the evolution of polyandry, thereby limiting the potential for divergence at the precopulatory stage.[17] Rather than relying on conventional phenotype-based mate choice, females may reduce the risk and/or cost of fertilization by genetically-incompatible sperm more effectively by mating with more than one male and exploiting the postcopulatory mechanisms of sperm competition, female choice of sperm and the reallocation of maternal resources from defective to viable embryos.[17,18] Such selection for polyandry and decreased reliance on precopulatory female choice within populations is likely to constrain the evolution of mating barriers between populations. This combination of rapid coevolution between embryos and mothers and restrained precopulatory divergence should amplify the importance of postzygotic isolation as a speciation mechanism among viviparous taxa.

Genetic and reproductive divergence in mammals

Our current understanding of the genetics of reproductive isolation has been based largely on comparative studies of *Drosophila*, with limited consideration of the potential implications of reproductive mode for speciation. In this section, we identify a number of features of divergence in mammals that are not adequately addressed within existing speciation paradigms. These patterns, however, do appear to be consistent with viviparity-driven conflict playing a fundamental role in the evolution of postzygotic isolation in mammals.

Feto-maternal interactions as a barrier to hybridization

Evidence suggests that incompatibility between embryo and maternal environment is frequently the primary postzygotic barrier to mammalian hybridization.[8,9,36–39] The rapid evolution of embryonic inviability in mammals provides only equivocal support for prevailing theories on the evolution of postzygotic isolation in animals.[3] These models propose that incompatibility evolves through beneficial or neutral genetic changes in one population that are deleterious when introduced into the genetic background of another population.

Deleterious effects are postulated to occur because negative interactions between genes from isolated (or partially-isolated) populations have not been screened by natural selection.[40] The theory predicts an evolutionary progression through hybrid sterility of the heterogametic sex to eventual hybrid inviability of both sexes, a trend well supported by comparative studies of *Drosophila*.[12] Turelli[6] has suggested that "Haldane's rule is a way station through which almost all pairs of animal species pass on their way to producing completely inviable or sterile hybrids." While it is true that, if only one sex from a mammalian interspecific cross is sterile, it is more likely to be the male,[36] in many cases, diverging mammalian populations appear to follow a direct path to embryonic inviability of both sexes.[41] High rates of fetal mortality in males and females, accompanied by production of few but fertile males, have been documented in several interspecific crosses in mammals.[36,39,42]

As a result of tissue-specific genomic imprinting, the maternal and paternal genomes play unequal roles in the formation of the embryo, the trophoblast and the placenta.[43] During the early stages of embryonic development, the trophoblast is the fetal membrane responsible for attachment of the embryo to the uterine lining, and, as such, is specialized for metabolic exchange, hormone production and nutrient acquisition from the mother.[44] Consistent with the conflict hypothesis for imprinting,[23,24] allelic expression in the trophoblast is predominantly paternal.[45] There is tantalizing evidence that deleterious interactions between this paternally-programmed extraembryonic membrane and the reproductive system of the mother can constitute a critical barrier to hybridization.[38] In crosses between *Mus caroli* males and *M. musculus* females, more than 99% of pregnancies fail, with most embryos dying within days of fertilization. However, reconstituted blastocysts, in which an *M. caroli* inner cell mass (the embryo proper) is combined with an *M. musculus* trophoblast, result in viable interspecific chimeras when gestated in *M. musculus* females.[38] Later in embryonic development, the physiological functions of the eutherian trophoblast are assumed by the placenta, which paradoxically is among the most rapidly evolving of mammalian organs.[46] Such rapid divergence runs counter to the generally highly conserved nature of both traits expressed early in development and traits buffered from the effects of the abiotic environment.[47] As Haig[24] argues, the evolutionary dynamics of conflict-driven "move and countermove mean that the details of maternal-fetal relations may diverge rapidly between lineages."

Polyandry and asymmetrical isolation

The viviparity-driven conflict hypothesis predicts that postzygotic incompatibility should evolve most rapidly between species which differ markedly in levels of polyandry (Fig. 2). Moreover, the paternal genome of the more polyandrous species should be more aggressive, which is likely to result in an elevated rate of spontaneous abortion but greater fetal/placental growth in the limited number of hybrid embryos that are viable. Although additional studies are needed, compelling support for this prediction is provided by research on closely-related *Peromyscus* species.[42,48] Crosses between males from the polyandrous *P. maniculatus* and females from the (essentially) monogamous *P. polionotus* result in a high incidence of spontaneous abortion, near-term fetal death or death of pregnant females during late stages of gestation. Surviving fetuses are 33% heavier at birth than either parental species and almost 200% heavier than the reciprocal hybrid.[42] Although the success rate of pregnancies from the *P. polionotus* male × *P. maniculatus* female cross is much higher, the small size of the resulting hybrids may render them noncompetitive. Striking asymmetry in the postzygotic compatibilities of a reciprocal cross is, in fact, a relatively common feature of mammalian hybridization, e.g., horse × donkey, goat × sheep, hare × rabbit, *P. maniculatus* × *P. leucopus* and *Mus musculus* × *M. spretus*.[36,37,48,49] Generally, the disparity is greatest in the development of the fetal membranes and the placenta. In the reciprocal *P. maniculatus* × *P. polionotus* cross, for example, placental weight differs more than five-fold.[48]

Disproportionate effects of imprinted genes

Although it is estimated that, in mammals, only approximately 100 genes, i.e., 0.1% of functional loci, are imprinted,[50] the viviparity-driven conflict hypothesis predicts that imprinted genes should frequently be involved in postzygotic incompatibility. In the first study designed specifically to examine the role of imprinting in postzygotic isolation, imprinted gene expression was found to be disrupted in hybrids between recently-diverged *Peromyscus* species.[51] A further intriguing example suggesting that imbalance in imprinted gene expression can bring about rapid change in postzygotic compatibility is provided by crosses between wild and laboratory mice. As discussed previously (Fig. 2), the *Igf2r* locus, which is expressed only by the maternal genome, encodes a receptor responsible for degrading the product of the paternally-expressed *Igf2* locus.[52] In laboratory mice, deletion of the maternal *Igf2r* causes embryonic lethality. Remarkably, however, embryos with the maternal *Igf2r* deletion survive and grow to a larger size than littermates, if sired by a male derived from a wild population.[53] More studies are needed to critically assess the relationship between divergence in imprinted gene expression and postzygotic incompatibility.

Viviparity and chromosomal evolution

At the level of chromosome and chromosome arm numbers, mean rates of karyotypic change, averaged over genera, have been estimated to be an order of magnitude higher in

mammals than in other vertebrates.[54] However, recent assessment of chromosomal evolution, based on comparative gene mapping and chromosome painting, suggests that such averaging obscures an intriguing pattern of highly conserved genomic organization punctuated by infrequent episodes of abrupt genome rearrangement in several mammalian orders.[55,56] Despite this rate heterogeneity, chromosomal evolution rate does appear to have been generally low in the early phase of mammalian diversification.[57] Moreover, comparison of monotreme, marsupial and eutherian mammals suggests a possible link between chromosomal evolution rate and the extent and duration of embryonic association with the maternal uterine environment. At one extreme, the ovoviviparous echidna and platypus monotremes[58] share almost identical karyotypes, despite more than 70 million years of independent evolution.[59] Among marsupials, chromosomal evolution rate appears to be positively correlated with proportion of embryonic development that occurs within the mother. Among the diverse dasyurids, for example, extreme karyotypic conservatism is associated with the production of highly altricial neonates.[58] By contrast, macropodids, such as the rock wallabies, give birth to relatively advanced young[60] and exhibit extensive karyotypic diversity.[59] Within placental mammals, the relationship is less clear cut.[54,58] Some eutherians do exhibit patterns consistent with a link between intensity of viviparity-driven conflict and chromosomal evolution rate (e.g., altricial young and chromosomal stasis in shrews versus longer intrauterine development, greater inter-embryo competition and rapid chromosomal evolution in mice). In other mammalian groups, such as Canidae, Ursidae and lesser apes, rapid rates of genome reorganization[55] do not appear to be attributable to intensification of viviparity-driven conflict. It is important to note, however, that comparative genomic data are currently available for only 11 of 28 mammalian orders.[55] Moreover, rearrangements detected by chromosome painting are mainly translocations and these represent only a fraction of the chromosomal changes that actually occur during evolution. In a recent study of various primates and outgroup mammals, subchromosomal probes detected four times more intrachromosomal rearrangements than the number of interchromosomal translocations revealed by chromosome painting alone.[61] Clearly, more studies of this type are needed to systematically investigate a possible link between intensity of viviparity-driven conflict and chromosomal evolution in placental mammals.

Limited evidence for F_2 breakdown in mammalian hybrids

In a number of *Drosophila* population/species pairs capable of producing viable F_1 hybrids, postzygotic isolation is manifested primarily as low success in the production of the F_2 generation.[62] This classic pattern of F_2 hybrid breakdown is generally attributed to the breakup of co-adapted gene complexes that occurs during meiosis in the F_1 hybrids.[63] The viviparity-driven conflict hypothesis, by contrast, would predict that the primary barrier to hybridization in mammals should often result from deleterious interactions between diverged maternal and paternal genomes in the developing F_1 hybrid embryo. Such deleterious interactions should be diluted by recombination during gametogenesis in the F_1 generation, resulting in increased viable F_2 offspring production. Such "F_2 hybrid enhancement" does, in fact, appear to be a general characteristic of mammalian hybridization.[36,39,42,64]

Previous hypotheses to explain between-lineage differences in speciation

Here, we discuss two previously proposed explanations for the rapid rate at which postzygotic isolation evolves in mammals relative to other vertebrates. Although these hypotheses are not mutually exclusive, they do make a number of alternative, testable predictions.

The regulatory gene hypothesis

In a pioneering series of studies, Prager, Wilson and colleagues proposed that, in birds and frogs, the capacity of distantly-related species to successfully hybridize was linked to a much slower rate of chromosomal evolution than that of mammals.[8,9] They hypothesized that karyotypic evolution involving gene rearrangements should generate abrupt evolutionary changes through modifications to gene regulatory systems.[7,10] Because individuals heterozygous for rare chromosomal rearrangements are likely to experience low fitness.[65] the rapid rate of karyotypic change in mammals was attributed to genetic drift acting in small populations. An association between social system and chromosomal evolution rate,[54,66] with the highest rates occurring in species with clan/harem mating systems or in territorial or patchily-distributed species, was taken as further evidence in support of drift-generated fixation of chromosomal rearrangements.[54]

The regulatory gene hypothesis was, of course, proposed before the discovery of the critical role played by parent-of-origin gene expression in mammalian development. This hypothesis would predict that hybrid disruption should manifest itself most strongly as a breakdown in the development of the morphogenetically complex embryo itself. By contrast, the viviparity-driven conflict hypothesis predicts that hybrid disruption should evolve most rapidly at the feto-maternal interface in tissues characterized by disproportionate paternal gene expression, i.e., in the trophoblast and placenta. The two hypotheses also make distinct predictions regarding the factors responsible for variation in rates of evolutionary divergence. According to the regulatory gene hypothesis, ecological and/or social system determinants of effective population size govern divergence rates, whereas

level of polyandry is most critical in the case of viviparity-driven conflict.

Although there is widespread support for drift-generated chromosomal evolution,[57] mathematical models and empirical data suggest that mammalian effective population sizes may often exceed those required for fixation of chromosomal rearrangements.[67] An alternative explanation is that changes in chromosomal architecture have significant effects on imprinted gene expression that, in rare instances, confer a competitive advantage. Although evidence is limited, patterns in the evolution and expression of imprinted genes suggest that this hypothesis warrants investigation. Imprinted genes are functionally haploid and deleterious mutations are likely to be rapidly eliminated by selection,[27] which may explain why such genes exhibit low rates of nucleotide substitution.[68] However, imprinted gene expression can vary dramatically as a consequence of modifier loci, with little or no change in the nucleotide sequence of the imprinted alleles themselves.[35,69] Moreover, many imprinted genes occur in chromosomal clusters, i.e., they are closely linked, e.g., *H19, Mash2, Kvlqt1, p57, Igf2* and *Ins2*.[70] Because control of imprinted gene expression is a complex process involving both cis- (methylation, direct repeat clusters and antisense and non-translated RNAs) and trans-acting (methylase and chromatin) factors.[71] chromosomal rearrangements are likely to profoundly affect imprinted gene expression.

The immunological hypothesis

As is evident from the burgeoning field of reproductive immunology, intolerance of the embryo, as an antigenetically-foreign body growing within the female, can represent a significant barrier to reproduction even within an apparently genetically cohesive species. In humans, for example, it is estimated that 70% of conceptions fail.[72] Similar estimates of early fetal loss, ranging from 10% to 60%, have been obtained for other mammalian species.[73] In human couples experiencing primary recurrent spontaneous abortion, approximately 55% of cases have been linked to chromosomal abnormalities in the embryonic trophoblast, with the remaining 45% attributed to immunological factors.[74] These data suggest that disrupted feto-maternal immunological interactions could well be a critical factor isolating mammalian species at the postzygotic stage.

It was initially supposed that experimental elimination of possible immunological barriers to hybridization could be achieved simply by suppressing the female's immune system. The failure of these early immunosuppressive experiments led Wilson et al.[8] to reject the immunological hypothesis. However, with considerably more now known about reproductive immunology, it seems that this rejection was premature. There is growing evidence that viviparous reproduction depends on a complex sequence of two-way interactions between fetal and maternal tissues that involves humoral, cellular, innate and adaptive immune responses.[72] There is currently extensive support for the view that immune tolerance is established through innate immunological interactions between maternal uterine natural killer (NK) cells that produce pro-inflammatory T-helper 1 (Th1) cytokines and trophoblast cells that react by triggering a T-helper 2 (Th2) anti-inflammatory cytokine response. Complement system regulation[75] and shift in balance from Th1 to Th2 cytokines are thought to be critical in determining the success or failure of pregnancy.[76] Also implicated in modulation of maternal NK cell-mediated attack is *HLA-G* (human leucocyte antigen-G), a non-classic MHC (major histocompatibility) class I gene of limited polymorphism, which is paternally expressed in trophoblast cells, primarily during early fetal development.[77] *HLA-G* is known to protect melanoma cells from NK cytolysis.[78] At the classic, highly polymorphic MHC loci, expression is minimal immediately following implantation but increases steadily through pregnancy in placental and extravillous cytotrophoblast tissues.[79] Restriction of polymorphic MHC gene expression to later stages of embryonic development probably serves to limit maternal rejection of the fetus. Paradoxically, however, MHC haplotype sharing between mates results in increased rates of spontaneous abortion in humans[80] and other primates.[81]

At the interspecific level, immunological treatments can markedly improve the female's capacity to successfully gestate "foreign" embryos in certain extraspecies pregnancies.[49] For example, pregnancy success rate of donkey embryos gestated in female horses is significantly enhanced by: (1) infusion of serum from mares carrying normal intraspecies horse pregnancies, or (2) immunization of recipient mares with donkey peripheral blood lymphocytes. [49] While further studies are necessary, these findings suggest that breakdown in the immunological détente between mother and hybrid embryo may, in some cases, significantly contribute to the rapid evolution of postzygotic isolation in mammals.

Does the immunological hypothesis represent a distinct alternative to the viviparity-driven conflict hypothesis? We suggest that in many cases it does not. Immunologically based postzygotic isolation could, in principle, result entirely from immune system divergence in response to differences between populations in exposure to environmental factors such as pathogens. However, maternal immune reactions are directed largely against paternal antigens expressed by genomically-imprinted genes in the trophoblast and placenta. Immunologically mediated feto-maternal incompatibility between populations may therefore frequently be a consequence of, rather than an alternative to, viviparity-driven conflict.

Non-mammalian, viviparous taxa

Although the overwhelming majority of non-mammalian species are oviparous, viviparity has evolved independently

numerous times in a diverse range of animal taxa, including echinoderms,[82] arthropods,[83] fishes,[84,85] amphibians[86] and reptiles.[87] The forms (and, indeed, the definitions) that viviparity can take are equally diverse, with great variation in extent of physiological interaction between mother and embryo. For example, in flies (Diptera), at least 61 independent origins of viviparity have recently been identified.[88] Interpreted literally as "live birth," viviparity in Diptera is defined as a reproductive mode in which the larva hatches within the female before deposition. The term is thus applied not only to species in which larvae are nourished by maternal glandular secretions (pseudo-placental) but also to species with embryos nourished exclusively on egg yolk (lecithotrophic). Similarly, in lizards and snakes, placental viviparity is believed to have originated at least 100 times.[87] However, in many species, embryos implant relatively late in development and in a superficial manner compared to mammals.[89] Consequently, the potential for viviparity-driven conflict may be limited. Although maternal input has evolved in some shark and ray species,[85] viviparity in elasmobranchs generally involves provisioning with egg yolk only.[90] Clearly, then, testing the viviparity-driven conflict hypothesis in non-mammalian taxa will require careful assessment of the extent of maternal/embryological physiological interaction in species classified as viviparous. In fact, the potential for between-embryo competition and feto-maternal conflict may be greater in some arthropod taxa described as ovoviviparous. For example, pseudoscorpions exhibit an "external womb" form of viviparity in which embryos develop in a brood sac overlying the female's genital aperture and rupture vitelline membranes early in development. As many as 100 simultaneously developing embryos actively draw nutritive fluid from the mother's reproductive tract.[91] Interestingly, in the pseudoscorpion species complex, *Cordylochernes scorpioides*, postzygotic reproductive isolation has evolved at a rapid rate in the absence of appreciable morphological or behavioral differentiation,[92,93] as is the case in many placental mammals.

Testing the viviparity-driven conflict hypothesis

Comparison of patterns of reproductive divergence in mammals, egg-laying vertebrates, and *Drosophila* provides tantalizing support for the hypothesis that reproductive mode exerts a strong influence on the relative rates at which pre- and postzygotic isolation evolve. However, additional studies of population pairs spanning a broad range of evolutionary divergence are needed to critically assess the effect of viviparity-driven conflict on the evolution of reproductive isolation in mammals. In particular, these studies should focus on the extent to which hybridization is limited by divergence at either the mating or the gametic stage of reproduction or by post-fertilization, feto-maternal incompatibility. In addition, far more information is needed on the importance of imbalance in imprinted gene expression in postzygotic isolation. Finally, it should be pointed out that a data set confined to a single viviparous lineage lacks the replication needed for a general assessment of the role played by reproductive mode in speciation. Although studies to quantify relative rates of divergence across the mating, gametic and postzygotic stages of reproduction have been carried out on a range of oviparous taxa,[3] investigation of speciation patterns in viviparous animals has, to date, been performed almost exclusively on placental mammals. Ideally, sister-group contrasts[14] of clades differing in reproductive mode would provide the most rigorous comparative method for testing the viviparity-driven conflict hypothesis. This approach would require data on relative rates of pre- versus postzygotic divergence in lineages comprising both viviparous and oviparous species. Alternatively, experimental evolution studies could be conducted on species with short generation times, such as mice or pseudoscorpions. These long-term selection studies would involve manipulating mating system, for instance, through the imposition of strict monogamy on laboratory lines of a naturally polyandrous species, to assess the effect of varying levels of inter-embryo competition and feto-maternal conflict on the evolution of postzygotic reproductive divergence.

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