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Christopher Tossing
Lake Forest College

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Speciation: The Genetics Responsible for Intrinsic Post-Zygotic Isolation

Christopher Tossing*

Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Intrinsic post-zygotic isolation (sterility or inviability of hybrids between two species) is an intriguing, yet complex component of speciation. While the effects of post-zygotic isolation have been observed for years, the direct cause remains unclear. However, advances in modern genetics elucidate some of the questions surrounding both the cause and function of post-zygotic isolation. By using *Drosophila* as the ideal model, researchers are able to characterize and identify genes that may be responsible for both the hybrid sterility and inviability associated with this type of isolation. Knowing which genes are responsible for these types of incompatibilities has also led to a better understanding of how and why they changed and how they result in speciation.

Introduction

One of the great complexities of evolution is describing the way in which new species form. Describing what defines a species is difficult enough. Even Darwin, the "father of evolution," found defining species difficult. Darwin stated: "... I was much struck how entirely vague and arbitrary is the distinction between species and varieties" (Darwin, 1859). Therefore, if differentially defining species is difficult, it is just as difficult to describe how species occur. The process of forming two species is called speciation. Speciation is formally defined as the evolutionary formation of a new biological species, usually driven by the divergence of one species into two or more genetically distinct ones. Speciation can occur by many different mechanisms, including physical separation (due to geographical location), behavioral isolation (which occurs when two species are not attracted to each other), and mechanical isolation (which refers to the incompatibility of reproductive organs) (Coyne & Orr, 2004). However, two species may also be unable to reproduce even if they are able to mate. This is the case with post-zygotic speciation. There are two types of post-zygotic speciation: extrinsic and intrinsic. Extrinsic post-zygotic speciation occurs in the offspring after two species have mated and generally results in offspring that have low fitness in the environment. Low environmental fitness simply means the offspring do not fit in as result of being an intermediate of two species. Offspring in this case either cannot find mates because they do not look like either of their parents or they cannot find an ecological environment that suits them. The second type of post-zygotic speciation is intrinsic, which occurs during the actual creation of the offspring. Intrinsic post-zygotic speciation results in hybrid offspring that are either inviable or sterile, which prevents them from being able to further reproduce and continue the common gene pool (Coyne & Orr, 2004).

It has been determined that inviability and sterility seen in the hybrid offspring stem from a divergence or mutation in a single gene or multiple genes. In some cases,

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divergence of two species results from a gene completely changing chromosomes, called gene transposition (Presgraves & Stephan, 2006). These mutations occur during periods of allopatry when the species are separated. No gene exchange occurs between the two species during allopatry. When these mutations develop, the individual species remain unaffected. However, when the two species are brought in contact with one another and try to reproduce, their offspring often have difficulties because some of their genes have become incompatible. As a result, the offspring themselves either do not survive (inviability) or cannot reproduce. The development of this type of speciation can take a long time, and often the cross between two closely related species results in offspring that are incompletely sterile or inviable (Conye & Orr, 2004).

The incomplete sterility and inviability of the hybrid offspring suggests that genetic isolation is an active process of speciation and not just a static barrier. Intrinsic post-zygotic speciation arises by many different mechanisms The elucidation of these mechanisms will lead to a better understanding of how these genetic barriers occur and aid in the development of complete speciation.

Dobzhansky-Muller Model

The Dobzhansky-Muller model takes an important place in the history of speciation genetics because it explains how two genotypes could be derived from a common ancestor, and yet when they are recombined, could produce a detrimental effect. Dobzhansky (1934) and Muller (1939) independently derived a model that could explain this phenomenon (Coyne & Orr, 2004). They proposed a model that begins when two genetically identical populations become separated geographically or become allopatric. While these populations are in their separate environments, they experience adaptive selection. Adaptive selection is the acquisition of new alleles or mutations acquired over time that enable the species to be better adapted to their environment (Orr, 1995).
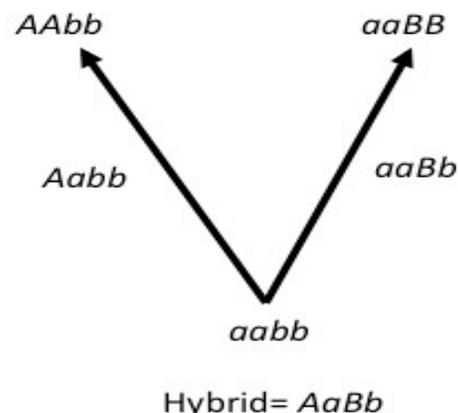

Figure 1. (Modified from (Coyne & Orr, 2004))

The two populations start with identical genotypes in two interacting alleles (aa,bb). After a period of separation, one population develops an A allele by mutation and it becomes fixed in the population; the Aabb and AAbb genotypes are perfectly viable and fertile. While at relatively

the same time in the other population, a B mutation appears and becomes fixed; aaBb and aaBB are also viable and fertile. The alleles A and B have never been in the same population. If the two populations meet and hybridize, the combination of the A and B alleles could result in a negative interaction leading to sterile or inviable offspring. This type of reproductive isolation results from an interaction (also called epistasis) between the A and B alleles (Presgraves, et al., 2003). However, this negative interaction does not necessarily lead to complete sterility or inviability; it may also exhibit incomplete dominance, meaning when combined they express a phenotype that is intermediate between the two alleles (Orr, 1995). It is also entirely possible that adaptive selection has occurred at several other locations on the genome, and therefore the cumulative effect of these incompatibilities may lead to complete sterility or inviability. It appears, at least initially, that almost all hybridization effects have occurred due to functional incompatibilities between two or more diverged loci (Coyne & Orr, 2004).

Several studies suggest that one of the incompatibility loci is usually located on the X chromosome. There are several theories that attempt to explain why this might be the case. Some believe incompatibility loci are located on the X chromosome because it is the only one that can be hemizygous. In other words, the X chromosome is able to exist without its pairing, homologous chromosome. This is exhibited in males since they only possess one X chromosome (Pregraves & Stephan, 2006; Phadnis & Orr, 2009). Others believe that the X chromosome evolves faster than other chromosomes. These theories are investigated further in the following section, as they are used to explain one of the fundamental theories behind speciation genetics: Haldane's rule.

Haldane's Rule

The formation of Haldane's rule in 1922 started as an observation and then became a significant finding in achieving a better understanding of speciation genetics. Haldane's rule states: "When in the offspring of two different animal races one sex is absent, rare, or sterile, that sex is heterozygous [Hetergametic, i.e. XY or ZW] sex" (Coyne & Orr, 2004). This rule has several important consequences. First, this rule suggests that incompatibilities resulting from hybridization are linked closely to sex chromosomes, rather than just sex, because of the ability to be exhibited in just one sex. If the incompatibility stemmed from an autosomal deficiency, it would affect both sexes equally (Coyne & Orr, 2004). Also, this rule seems to suggest an early stage of evolutionary divergence because if the incompatibilities are only in one sex, there is enough common genetic heritage to leave the other sex unaffected. It has now been found that Haldane's rule is obeyed almost universally in all organisms that possess sex chromosomes. This finding is significant because it implies that many organisms share similar genetic problems when they are attempting to hybridize with another genetically distinct species (Coyne & Orr, 1989). While the implications of this rule are fascinating, the real interest lies in discovering what causes the rule to hold true in so many species. No exact cause for Haldane's rule has been determined, but several theories have been proposed.

Dominance Theory- The first of these theories is the dominance theory. The dominance theory suggests that all hybrid males will be affected by genetic incompatibilities linked to the X chromosome. This is due to the fact that the X chromosome will always be exhibited as dominant in hemizygous males (Olsson, et al., 2004).

Figure 2 provides an example of the dominance theory. For instance, if there is a recessive allele on the X_1
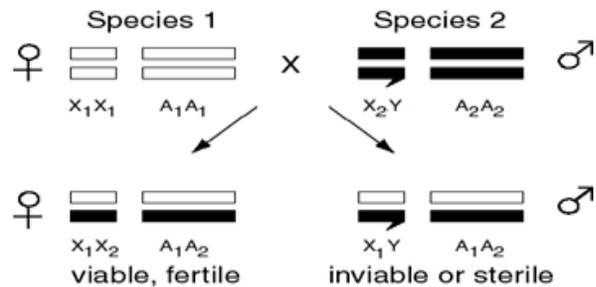

Figure 2.

chromosome that is incompatible with the autosomal chromosome A_2 allele, the resulting hybrid male will be either sterile or inviable depending on the type of interaction. However, this effect is not seen in the female because the presence of the X_2 chromosome compensates for the recessive incompatibility alleles, which prevents the negative effect from being exhibited. In order for an effect to be exhibited in the female, the X_1 and A_2 alleles would have to be dominant (Olsson, et al., 2004).

Meiotic Drive
Meiotic drive is another theory that could be partially responsible for the observation of Haldane's rule. Meiotic drive theory suggests there are specific genetic alleles that act to distort Mendelian ratios to their own benefit (Orr & Irving, 2004). Mendelian inheritance says that alleles on homologous (or paired) chromosomes have an equal chance of being inherited, so the ratio of inheritance of two alleles would be assumed to be 50:50. However, when the alleles exhibit meiotic drive they increase their chance of being inherited and thus are present in greater than 50% of the offspring. This occurs consistently and removes any possibility of random chance. It is believed that these meiotic drive genetic elements are often maintained within a species but remain suppressed. Yet when that species is hybridized with another species, the resulting hybrids do not possess the correct suppressors. Therefore the hybrids express selfish genetic elements. These selfish genetic elements are thought to function by inactivating the sperm carrying the homologous chromosome. These meiotic drive elements have been found expressed in the X chromosome in some species of *Drosophila* and could be present in other species as well. In these cases, the X chromosome is significantly over-represented, which results in mostly female hybrid offspring in the F_2 generation. In some cases, more than 90% of the hybrid offspring is female. When the X chromosome carries the meiotic drive element, it is thought to function by inactivating the sperm carrying the Y chromosome, which results in the hybrid offspring being mostly female (Orr & Irving, 2004).

Both of the significant theories behind speciation have now been explained. The first theory, the Dobzhansky-Muller model, established how new species arise from a common ancestor by acquiring mutations that prevent them from reestablishing a common genetic pool. The second theory, Haldane's rule, is based on the observation that the heterozygous sex is affected when two species have impartial incompatibilities because they are hemizygous for the X chromosome. These theories are incredibly important, but possibly more significant is how these theories are applied in actual models. The common model for these speciation studies is *Drosophila*, because they are easy to grow and maintain in the lab, and because this species has been researched in depth. The goal of the case studies in *Drosophila* is to develop a better understanding of how and

why the theories are observed. This is generally done by first establishing the outcome of hybrid crosses between two species and then by investigating the genetics behind it. A great deal can be learned about the mechanisms that cause speciation by studying the genes responsible for this process.

Studies in *Drosophila*

Approximately 155,000 to 230,000 years ago, two *Drosophila pseudoobscura* subspecies diverged: *Drosophila pseudoobscura pseudoobscura* (USA) *and Drosphila pseudoobscura bogotana* (Bogota).(Phadnis & Orr, 2009). The Bogota subspecies inhabits high elevations in Bogota, Columbia and are completely geographically isolated from the USA subspecies, which only inhabits North and Central America (Phadnis & Orr, 2009). The isolation means that these species do not hybridize in natural settings; therefore, their gene pools are completely separated from one another. However, because of their relatively recent divergence, they have not become completely reproductively isolated. When hybridized, these two subspecies produce completely fertile hybrid females. Also, when a male Bogota is hybridized with a female USA, they produce fertile males. The reciprocal cross with a female Bogota hybridized with a male USA produces males that are only weakly fertile when aged. Even when the hybrid male offspring (F_1 male) of the Bogota female and the USA male do produce offspring (F_2 generation), they produce almost all daughters. This is both an example of Haldane's rule and meiotic drive because it is only the male offspring that are being affected and the F_2 generation is exhibiting meiotic drive by the X chromosome (Phadnis & Orr, 2009).

In a study done by Orr and Irving, an attempt was made to see if the fertility of these hybrids could be rescued (2004). Finding a gene that could recover the fertility of the male hybrid could be extremely important in understanding the genetics behind fertility. Their study showed that specific mutation on the USA second chromosome, labeled *Hybrid male fertile* (*Hmf*), contributes to the weak hybrid fertility seen after several weeks without this mutation, the F_1 males are completely sterile (Orr & Irving, 2004). Essentially, some factor of this gene allows the hybrid males to overcome their sterility once the *Hmf* gene or the *Drosophila* itself have matured. The mechanism behind this gene's ability to rescue fertility is not known. However, even with the *Hmf* mutation, the sex ratios are still almost completely female (typically, more than 90%), indicating that *Hmf* does not play a role in the sex ratio distortion. The sex ratio distortion was predicted to be caused by a meiotic drive element (generally referred to in these cases as segregation distortion) in the X chromosome of the Bogota (Orr & Irving, 2004). This is a valid prediction because the F_1 males get their X chromosome from the Bogota female and it is the X chromosome that is being passed from the father to the daughters. This expression of meiotic drive means that some gene or genes on the Bogota X chromosome seems to be interacting with a gene from the USA in a way that significantly decreases the inheritance of the F_1 males Y chromosome, thereby decreasing the number of males produced (Orr & Irving, 2004).

Another study by Phadnis and Orr was able to identify the specific gene on the Bogota X chromosome responsible for not only the sex ratio distortion, but the F_1 male sterility as well (2009). This study was groundbreaking due to the fact that they found that one gene can be responsible for both distortion and sterility. This finding indicates that distortion and sterility could possibly be linked. In order to discover this they used introgression lines. This process involves the movement of a gene region from one

species to another by mating a hybrid with one of the pure parents. This is done to narrow down the possible interactions that could be causing the sex ratio distortion. Using this procedure, they removed the region that they thought was responsible for the negative effects from the Bogota female and replaced it with the same region from the USA. When they crossed the new Bogota female with a normal USA male, there was no distortion or sterility, indicating that a gene in this region was responsible. They then took a specific gene, GA19777, from the Bogota and inserted it into the USA. In result, the males produced from this cross were sterile and produced offspring that were primarily female. This indicates that the GA19777, named *Overdrive* (*Ovr*), from the Bogota X chromosome, is responsible for the sterility seen in the F_1 males and the sex ratio distortion seen in the F_2 generation (Phadnis & Orr, 2009). This is a perfect example of Haldane's rule and meiotic drive. The Bogota X chromosome gene only has negative effects in the male. This signifies that negative effects result from not having another X chromosome, since the presence of the USA X chromosome in the female hides its effects. Also, the Bogota X chromosome gene seems to have a negative impact on the inheritance of the USA Y chromosome because almost all the F_2 offspring are female, meaning they only inherit the Bogota X chromosome. Before this study, many thought that it would take multiple mutations to exhibit one of these effects, but no one imagined that one mutation could be responsible for both. This is significant because it is now known that a change in a single gene can lead to dramatic progress towards speciation. This finding also opens the possibility that, in some species, it may be only one gene that keeps them completely separate (Phadnis & Orr, 2009).

Drosophila melanogaster and Drosophila simulans
The *D. simulans* diverged from the *D. melanogaster* approximately 2 to 3 million years ago (Presgraves & Stephan, 2006). Over this time, these two species have acquired several incompatibilities at different loci. Two of these incompatibilities are significant because they both seem to occur in functional genes, meaning that they occur in genes that are critical to survival and that play an important role in cellular development. This is opposed to incompatibilities that occur in genes that are parasitic or do not function in a specific capacity. The first, *JYAlpha* (codes for a Na/K ATPase) is essential for male sterility (Presgraves & Stephan, 2006). This gene is interesting because the incompatibility is not caused by a mutation; instead it is caused by a gene transposition (changing chromosomes). In the *D. melanogaster*, the *JYAlpha* gene is on the third chromosome, but in the *D. simulans* it is on the fourth chromosome. Therefore, this incompatibility is not necessarily due to a mutation. Instead, the chromosomes just do not match up because *D. melanogaster* has an extra gene on chromosome three that *D. simulans* does not have, and *D. simulans* has an extra gene on chromosome four that *D. melanogaster* does not have. Thus, when hybridized together, they do not have a functional gene because the pair of genes are separated. This leads to sterility in all of the male hybrids. The second incompatibility is caused by a mutation in the *Nup96* gene from an autosomal chromosome from the *D. simulans*. The *Nup96* codes for a nuclear pore complex protein and causes inviability of males bearing an X-chromosome from the *D. melanogaster*.

The *Nup96* case is especially interesting for several reasons (Presgraves & Stephan, 2006). First, nuclear pore complex proteins are essential for proper cell structure and the sequences are highly conserved (relatively unchanged) throughout eukaryotic evolution. Second, in the presence of the *D. simulans* X chromosome there are no

adverse effects for the resulting hybrid offspring. This indicates that *Nup96* has a negative interaction with the X chromosome of the *D. melanogaster*, as well as the fact that in the *D. simulans* it had to be accompanied by a co-evolution of several of its interacting proteins. If the interacting proteins of *Nup96* did not co-evolve, the *D. simulans* would not survive because the nuclear pore complexes (which are essential to survival) would not form correctly and would lead to the inviability of *D. simulans* (Presgraves & Stephan, 2006). The divergence of an essential gene like this leads to an important question: What leads to the development of these incompatibilities, especially in an essential, highly conserved gene like *Nup96*?

Causes of Mutations

In a topic shrouded with unknowns and complicated problems, it is no wonder that the question of what drives these genetic divergences and how they originated is equally ambigious and complex. Some of these incompatibilities between species are driven by random mutation and ecological adaptation. In two isolated populations it is entirely possible that, like the Dobzhansky-Muller model, these incompatibilities arise by a simple random mutation at a critical point in the genome and then become fixed within the population. As demonstrated by the *Drosophila* models, these mutations can be in one gene or in many in order to cause genetic incompatibility. How do these mutations become fixed within a population? There are two possibilities: genetic drift and adaptive evolution.

Genetic drift is the slower of the two processes because it requires the build-up of random mutations which occur frequently enough to become fixed in the population. These mutations take an extremely long time to become fixed in a population because they form by random chance, and the resulting infrequency requires an extended period of time for enough mutations to build up to make two populations incompatible. This could be why, for example, the *Drosophila* USA and Bogota have been separated for about 200,000 years and are only incompatible in one specific cross. As long as their gene pools remain separated over time, they will continue to build up mutations that make them incompatible.

The second possibility occurs if the mutation ends up resulting in beneficial adaptation for the organism. The fixation within the population can be driven by natural selection, which in this case is called adaptive evolution. The organism can undergo adaptive evolution to better adjust to their external environment. For instance, this process can help an organism co-exist with a new plant or survive a a change in climate. These mutations become fixed faster than simple genetic drift because they have a driving force. Organisms with these mutations have some benefit, such as increased attractiveness to the opposite sex or better survival. However the mutation occurs, they would most likely exhibit no ill effects on the *Drosophila* until brought in contact with another species that did not acquire the same mutations or with different species that are incompatible.

There are other ways that incompatibility can be driven other than genetic drift or adaptive evolution to the extrinsic environment (Presgraves & Stephan, 2006). One of these examples is the *Nup96* gene in the *D. simulans* and the *D. melanogaster*. It is hypothesized that this mutation could not be driven by adaptive selection to the environment. In order for this to be the case, both species had to undergo an environmental challenge at roughly the same time. Not only that, but the environmental challenge had to pose a challenge to the same set of genes, so the chance of this being the case is highly unlikely. Instead, researchers

believe that both species inherited a type of deficiency involving the *Nup96* and interacting genes (Presgraves & Stephan, 2006). This would cause both species to form their own independent solutions to the same problem, thus leading to different adaptation and eventually to their divergence. Several of these scenarios have been proposed. *Genetic Conflict with Pathogen*- While not much is known about the viral immune response of *Drosophila*, it is proposed that they use strategies similar to humans. In humans, *Nup96* is up-regulated in reaction to viruses as part of a generated immune response. It is believed that in response to these viruses, *Drosophila* had to alter their *Nup96* gene, along with other related nuclear pore complex proteins, in order to better adapt themselves (Presgraves & Stephan, 2006). There are multiple solutions to dealing with the presence of a pathogen. Any new genetic development that can decrease the impact of the virus is beneficial to the *Drosophila*, and it is the independent arrival at two distinct solutions that leads to speciation. In one species, the *Drosophila* could change the structure of its *Nup96* protein so that it can better deal with the presence of a virus. On the other hand, another species could just change the receptors on the protein so that a specific virus cannot bind and cause harmful effects. Both solutions solve the problem of the virus, but they also lead to incompatibility between the two species because now one species has a different structure in their *Nup96* and the other has different receptors.
Genetic Conflict with Centromeric Drive- Centromeric drive is considered the meiotic drive of centromeres because they act selfishly, much like the genes in meiotic drive (Presgraves & Stephan, 2006). Centromeres are located at the center of a chromosome and generally serve as the point of attachment for sister chromatids. They are also involved in meiosis when the two sister chromatids are separated. Centromeric drive is thought to manifest during oogenesis, the female meiosis. During oogenesis, four products are created: three polar bodies and one primary oocyte, or egg cell. This creates unbalance because only the primary oocyte is able to be fertilized. Therefore, centromeres are able to compete for insertion into the primary oocyte. The exact mechanism of how these centromeres act selfishly is unknown, but it is believed it may influence the way centromeres bind to the spindles during the separation of chromosomes.

While the cause of centromeres' selfish behavior is unknown, the adverse effects of centromeric drive have been identified. The main side effect of centromeric drive is an increase in instances of non-disjunction, which occurs when either of the homologous chromosomes fail to separate during meiosis I, or when the sister chromatids fail to separate during meiosis II (Presgraves & Stephan, 2006). The nuclear pore complex proteins (*Nup96* and others) are believed to associate with the centromeres and their binding to spindles during meiosis I and II. Therefore, evolving these proteins to help suppress centromeric drive would be beneficial to both species (Presgraves & Stephan, 2006). However, just like the solution to the virus theory, there would be no absolute right answers and both species could arrive at a solution by different adaptations.

While both these possibilities are plausible, nothing can be determined for certain as to how or why the divergence in *Nup96* occurred. Right now, all theories are based on the overall role of *Nup96* and its function in the cell. However, the answer to how and why the gene changed could lie in the smaller subtle differences between the *Nup96* gene in the two species. The smaller differences in DNA or amino acid sequence could change many things, such as structure. Identifying specific differences in the structure could help researchers pinpoint what exactly was changed

and its function, which could lead to a better guess as to why it changed.

Conclusion

In order to begin to understand intrinsic post-zygotic speciation, one must first understand the theories behind it. The first theory, the Dobzhansky-Muller model, attempts to explain how species arise through geographic separation and eventually lead to a build-up of genetic incompatibilities. The second theory, Haldane's rule, illustrates that when one sex is more drastically affected it is the hemizygous sex. This has important implications in the genetics of speciation because it seems to point out that many of the incompatibilities seen in hybridization arise from the X chromosome. The genetic studies in *Drosophila* seem to support this observation because at least one of the crosses in the USA, Bogota, and *melanogaster* x *simulans* results in the male offspring being sterile, inviable, or exhibiting meiotic drive. Researchers have also linked at least one gene, *Ovr*, on the X chromosome to these negative outcomes exhibited in the hybrid offspring. Another gene, *Nup96*, is also indicated to have a negative interaction with the X chromosome, which leads to the same negative outcomes. These two examples are only a glimpse into the genes responsible for divergence and negative outcomes for hybrids. While these genes and their interactions are informative, as well as interesting, they only contribute to half of the puzzle that is speciation. The other half of the speciation puzzle is how these incompatibilities arise. This question is often more difficult to answer and requires much more speculation because it frequently lacks concrete evidence. Most of the incompatibilities probably arise from genetic drift or adaptive evolution. However, there are interesting cases like *Nup96*, which seems to suggest co-evolution of two species in response to a common inherited flaw. This is just one example of the many ways and reasons speciation can occur but there are many more possibilities, which makes speciation very hard to define and predict.

Intrinsic post-zygotic speciation is an extremely complex topic to study. First of all, there is very little concrete evidence to consider. Secondly, once speciation has occurred completely, the resulting offspring are sterile or inviable, which limits the ability for in-depth study of the factors involved at the point of divergence. However, with the increasing technological advances being made in genetics, it may only be a matter of time before we can more accurately assess the genes involved in speciation. More knowledge about the genes, especially sequence and function, will lead to better conclusions about how, and possibly even why, they diverged.

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