

Timema cristinae

Lorraine Scanlon*

Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Speciation is responsible for the vast biodiversity seen on earth today. Over time, natural selection allows for organisms to evolve traits and characteristics that make them a better fit for their niche or habitat. In this way, natural selection may cause two groups derived from a single species to differ progressively over generations as different traits evolve in each group. When enough difference is observed between the divergent populations, a new species is formed. This process allows for the considerable types of diverse organisms that inhabit the earth today.

Although it may seem simple, the study of speciation is quite difficult. There are several theories that attempt to clearly define when speciation occurs within a divergent population, however nature does not allow such a clear cut definition to hold true in all cases. Because speciation may occur on a time scale anywhere between a few years to thousands of years it is almost impossible to determine the exact point at which a new species is formed. The point of speciation is also made difficult to determine when one considers the several ways in which new species are formed through divergence from a parent population. Generally, it is agreed that between two species there must be a certain degree of genetic, phenotypic, ecological, and behavioral differentiation so that the populations are not able to successfully interbreed and hybridize. According to this definition, one organism that appears to be on the edge of speciation is *Timema cristinae*, walking stick bugs.

Timema cristinae is a species of wingless phytophagous insects that inhabit the chaparral of southwestern North America (Crespi & Sandoval 2000). The insects rest on the branches or leaves of their host plant during the day and move around to feed and mate during the night. The insects usually feed and mate strictly on their host plant which is determined by which ecotype of *T. cristinae* they inhabit.

There are two ecotypes of *T. cristinae*: the *Adenostoma* ecotype and the *Ceanothus* ecotype. The ecotypes are named for the host-plant that they primarily inhabit: *Adenostoma fasciculatum* and *Ceanothus spinosus*. These plants are distributed in patches that create a mosaic of both parapatric and allopatric situations (Nosil & Crespi 2003). In allopatry, a species is separated by some sort of physical barrier that creates separate populations. In the case of *T. cristinae* the physical barrier is the uprising of plants that are not suitable hosts so that the insects living on a given host may not successfully migrate to another.

In parapatric situations, there is a large community of host plants which are in close enough range for the insects to switch hosts. In parapatry, the host plant population is large enough that insects living on the outermost edges of the population cannot travel completely to the other side. This allows for some interbreeding between insects living on adjacent plants, but there is not an overall mixing of populations from two host patches.

The two ecotypes of *Timema cristinae* differ very much phenotypically which can be easily observed in their body shape, body size, color, and the size/shape of their

head, depending upon their native host plant. *Adenostoma* is a small, bush-like plant with needle-like leaves, while *Ceanothus* is a tree-like, broad-leaved plant (Nosil 2007). The *Adenostoma* ecotype exhibits smaller body size, wide heads, short legs, and a white dorsal stripe when compared to the *Ceanothus* ecotype. Those resting on *Ceanothus* are predominantly green with no dorsal stripe (Nosil & Crespi 2004).

Because *Timema cristinae* is subject to predation by birds and lizards during the day, they employ cryptic coloration according to their host-plant (Sandoval & Nosil 2005). This cryptic coloration is genetically inherited and leads to sexual isolation and selection against hybridization as we will see. One would think that this would in turn lead to speciation; however, that is not yet the case for *Timema cristinae* walking stick bugs.

As a whole, *Timema cristinae* did not begin as two ecotypes that were specialized for separate hosts. In total, there are seventeen species of *Timema* some of which are highly specialized for one host-plant while others are generalized for up to four unrelated hosts (Sandoval, 1993). The earliest ancestors of *T. cristinae* appear to have been generalists who fed on both *Adenostoma* and *Ceanothus* among other plants (Crespi & Sandoval 2000). Phylogenetic evidence shows *T. cristinae* experienced divergent ecological specialization over a long period of time in order to form the two ecotypes seen today. This specialization has been driven by selection for reduced energetic costs in searching for a suitable host as well as predation. It has also been driven by selection against maladaptive host switching and between population gene flow (Nosil, Crespi & Sandoval 2002).

Ecological speciation occurs when an ecological source of divergent selection, such as a difference in niche, sexual selection, or the ecological interactions such as competition for resources of two populations, combined with reproductive isolation are linked by some genetic mechanism. These differences cause the two populations to diverge from one another. Within ecological speciation, barriers to gene flow develop between populations of a species due to ecologically-based divergent selection. This occurs when the interaction of individuals with their environment causes selection to act in contrasting directions due to competition for resource acquisition or attempting to avoid predators (Rundle & Nosil 2005). In the case of *Timema cristinae*, differences in niche, which are now determined by ecotype and were originally driven by competition for resources, have caused the insects to prefer mates of their own ecotype. This in turn has created reproductive isolation and is gradually leading the ecotypes towards speciation.

Descendant lineages of the *Timema cristinae* generalists slowly began to use a subset of the hosts of their ancestors. Divergent ecological specialization towards a specialized host preference continued to occur in each new generation. In each generation, the more specialized insects experienced higher levels of fitness because they were able to find suitable hosts easier and also to avoid predation more effectively. Due to the higher level of fitness enjoyed by these insects, selection continued to encourage specialization so that each successive semi-specialized generation produced offspring that were more and more specialized until they were completely specialized for either *Adenostoma* or *Ceanothus* (Crespi & Sandoval 2000). The same is true for color pattern as well.

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Due to ecological speciation acting upon their generalist ancestors, *Timema cristinae* have specialized host plant use, and color pattern. A study conducted by Crespi and Sandoval in 2000 considered the phylogeny of *Timema* as a species. Using mitochondrial DNA sequencing data, they constructed an evolutionary tree representing the evolutionary relationship among the species of *Timema*. Results showed that of the three main lineages that were identified in the phylogeny of *Timema*, *T. cristinae* and *T. nakipa* were the most generalized species for host-plant use in the genus (Crespi & Sandoval 2000). The same results were shown when considering the evolution of color pattern. This time, all three of the three main lineages of *Timema*, *T. cristinae*, *T. nakipa*, and *T. boharti* were found to exhibit three of the four possible color morphs found in *Timema* (Crespi & Sandoval 2000). These two studies combined show that *T. cristinae* is most closely related to the common ancestor of the entire species of *Timema*. This illustrates that their ancestors were highly generalized for host preference and color morph. However, as we see today, *T. cristinae* rests only upon two possible hosts and exhibits only two of the possible four color morphs. Although *T. cristinae* has the genes to be highly generalized, they are more specialized than their generalist ancestors.

As mentioned, this host preference specialization was partially driven by predation. As generalists, it was impossible for the insects to employ crypsis because of the wide variation of host phenotypes. This means that no insect phenotype would be cryptically colored for its host because the available hosts have so many different phenotypes. As the insects began to rest primarily upon a specific host, they were able to develop coloration that made it difficult for visual predators such as birds and lizards to detect them as they rest upon the leaves of their host. This adaptation is apparent when one considers the current differences in body size, body shape, and cryptic coloration between the two ecotypes (Nosil, Crespi & Sandoval 2002). These traits have evolved after host preference.

Selection against maladaptive host switching is also encouraged by predation (Nosil & Crespi 2006). In a study conducted by Sandoval and Nosil in 2006, it was shown that in the absence of predation, those mating on *Ceanothus* were able to lay more eggs regardless of their ecotype. In the absence of predation, *T. cristinae* insects were reared in the laboratory on their native host. The insects were either left on their native host or transplanted to the alternative host to mate. Independent of the insect's native host and whether it was left to mate on its native host, those mating on *Ceanothus* laid more eggs than those mating on *Adenostoma* (Fig. 1). This is possibly because *Ceanothus* is a better food source for females making it possible for them to lay more eggs.

However, there are survival benefits for those reared on their native host when compared to those not reared on their native host. In the same study conducted in 2006, Sandoval and Crespi demonstrated that in the presence of predation by birds, the higher fecundity experienced on *Ceanothus* was outweighed by survival trade-offs. Because *Timema cristinae*'s cryptic coloration allows for lowered levels of predation for those on resting on their native host plant, it was found that when the insects mated on the host that was not native to them, they suffered a higher level of predation (Fig. 1). When compared, the higher fecundity enjoyed when mating on *Ceanothus* could not compete with the higher level of predation if *Ceanothus* was not the insect's native host (Sandoval & Crespi 2006). Therefore, there is net selection for host specialization.

Specialization in *Timema* was also partially driven by selection for reduced energetic costs in searching for a suitable host. As generalists it was very energetically costly

for *T. cristinae* to find a suitable host (Crespi & Sandoval 2000). The lifecycle of *T. cristinae* begins when females drop eggs from the host upon which she is resting. These eggs remain in the soil during the winter. In January, the eggs hatch and the nymphs crawl to a host plant and generally remain on that host for the rest of their life (until June) (Bolnick & Nosil 2007). The eggs may either remain at the base of the host plant of their parent or they may be carried away by ants (Tilgner 2001). Before specialization, each individual insect would have to travel by crawling until they were able to find a host that was generally safe from predators that also offered the mating choices and nutrition necessary to survive. If this was not possible in a timely manner, the insects would die either due to starvation or predation. By specializing for a specific host, the insects may easily recognize the phytochemistry of their appropriate host and settle there. If the insects are not able to recognize their suitable host before they climb up it, they risk predation while on the plant and will also experience difficulty in finding a mate. Evolving a host-choice has taken the conjecture out of host selection thus greatly reducing the energetic costs of finding a suitable host (Crespi & Sandoval 2000).

Adenostoma and *Ceanothus* belong to different families with differing phytochemistry (Sandoval & Nosil 2005) which makes it much easier for the insects to recognize their suitable host. In 2005, Sandoval and Nosil conducted an experiment in which they placed insects in containers which included a cutting from either their native host or non-native host. It was found that individuals from both hosts preferred *Adenostoma* equally when placed in a container with *Adenostoma*. When placed in a container with *Ceanothus*, the *Adenostoma* ecotype did not choose a host while the *Ceanothus* ecotype chose *Ceanothus*. This shows that the *Adenostoma* ecotype is capable of recognizing the phytochemistry of their native host while the *Ceanothus* ecotype is not. This helps explain why despite all the differences in the ecotypes interbreeding and hybridization still occurs.

Since becoming specialized for either *Adenostoma* or *Ceanothus*, *T. cristinae* has evolved a significant degree

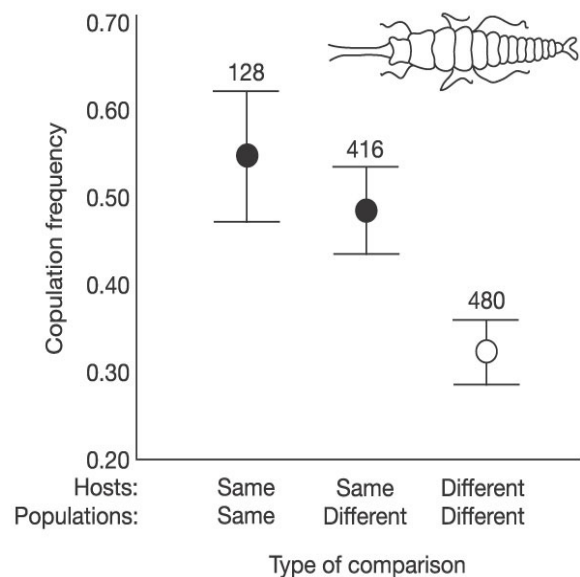


Fig. 1. Copulation frequencies were higher for ecologically similar insects than for ecologically divergent ones. These results suggest that reproductive isolation between populations has evolved due to selection for crypsis. (Nosil, Crespi, & Sandoval 2002)

of reproductive isolation. This reproductive isolation has been driven by ecological adaptation and reinforcement. Reinforcement is a process which requires that natural selection increases reproductive isolation in situations where hybridization is maladaptive.

Reproductive isolation includes a group of mechanisms that prevent two populations from exchanging genes. In *T. cristinae*, reproductive isolation is aided by the fact that the two ecotypes have separate host preferences. Host preference reduces gene flow between the ecotypes because they mate upon their chosen host. Reproductive isolation can also occur either by preventing fertilization, or by the creation of a degenerate or sterile hybrid. In *Timema cristinae*, reproductive isolation occurs between insects resting on *Ceanothus* and *Adenostoma*. Because these insects produce unfit hybrids when they mate, there is selection against hybridization which in turn leads to reproductive isolation. This assertion is supported by mate choice experiments. In 2002, Nosil, Crespi, and Sandoval conducted a study in which they gave the insects the choice between mate from their own ecotype and from the ecotype opposite their own. They found that copulation frequencies were higher for ecologically similar pairs of *T. cristinae* than for ecologically divergent pairs (Fig. 3). This in turn suggests that reproductive isolation has evolved due to selection for crypsis.

The selection for crypsis has also promoted selection against gene flow between the two ecotypes (Nosil, Crespi & Sandoval 2002). It has been shown that hybridization is maladaptive between ecotypes of *T. cristinae*. The cryptic coloration displayed by *T. cristinae* is genetically inherited which results in the creation of unfit hybrids when mating occurs between the two ecotypes (Nosil, Crespi & Sandoval 2003). Because cryptically unfit hybrids are created when the ecotypes mate, there is selection against gene flow between them.

This also creates sexual selection and isolation because during the mating period of *T. cristinae* the males ride on the back of the females (Nosil, Crespi & Sandoval 2003). If females choose to mate with males cryptically colored for another host or not cryptically colored for either host, they risk predation.

All of the above elements contribute to the divergent ecological evolution of *T. cristinae* from generalists to specialists. These elements have resulted in the evolution of a host-preference and in doing so have evolved two ecotypes. With all of the apparent differences between the ecotypes one may wonder why they are not considered two separate species. Speciation is almost complete in *Timema cristinae* however, reproductive isolation is not complete. Considering the original definition of speciation given above it demands that there must be a certain degree of genetic difference so that the populations are not able to successfully interbreed and hybridize. Although it has been shown that interbreeding between ecotypes is maladaptive, it does still occur. Such interbreeding produces gene flow between the two ecotypes of *T. cristinae* hindering speciation (Nosil, Crespi & Sandoval 2003).

Gene flow between the two ecotypes may result when juveniles choose the wrong host. Migration between hosts mainly occurs during the early stages of the life cycle of *T. cristinae*. As mentioned above, when a female drops her eggs into the soil they may remain at the foot of her host plant or be carried away by ants. When the eggs hatch, the nymphs will find the first appropriate host and begin to feed. If the eggs are carried to the alternate host they are not suitably colored to avoid predation on that plant making them maladaptive for their host. These maladapted insects rarely find mates but in some cases are able to do so.

Host switching and hybridization may also occur later in the life cycle of *T. cristinae* in parapatric situations. When this happens, mature adults will wander to the other host and mate there. This usually occurs in situations where there are insufficient mates available on the insect's given host (Nosil 2007).

The gene flow encountered when ecotypes breed with each other makes *T. cristinae* subject to a migration load (Bolnick & Nosil 2007). This means that the average fitness of the population is reduced when migration between hosts occurs. In these cases, migration may cause natural selection to reverse the direction of selection towards a more generalized species. As more and more insects are able to successfully hybridize, they continue to be able to find other hybrids like themselves with which to mate. Over time, this will cause each generation to be more generalized thus reversing the specialization that has already occurred. Overall, populations that are subject to a migration load are not threatening to reverse the specialization of *Timema cristinae* as a whole, but they are slowing the process of speciation.

Because allopatric populations of *T. cristinae* experience a higher level of reproductive isolation based on the fact that they do not encounter gene flow, they are not affected by a migration load (Nosil, Sandoval & Crespi 2006).

Generally, it is difficult to determine the direction and order of evolution within a species. In the case of *Timema cristinae* it appears that the species is headed towards two separate species based on host preference due to divergent ecological specialization. Those in parapatric populations, however, may experience host switching which makes them subject to a migration load. This in turn discourages speciation of the ecotypes. For these reasons, *Timema* is a great system for studying speciation. This is perhaps responsible for how well known *Timema* has become despite the fact that they are such obscure animals!

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