Chapter 16
Genetic Connectivity in Conservation of Freshwater Insects

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Abstract Ecosystems and species are disappearing fast and the conservation of isolated and fragmented landscapes is not enough to maintain healthy populations. However, populations from fragmented and impacted landscapes may be benefited if there are pathways allowing their connection. These pathways enable the exchange of individuals, allowing species to increase their genetic diversity and resilience to stochastic events by recolonization and phenotypic adaptations. In aquatic ecosystems, climate changes and water exploration are impacting the species' capability to disperse among populations and survival. In this scenario, aquatic insects are even more threatened as most of them have terrestrial and aquatic life stages, suffering impacts from both environments. Focusing in this aspect, this chapter aims to provide an initial insight about how population connectivity can be used in conservation strategies as well as methods of measuring genetic connectivity. Here we selected studies with odonates, ephemeropterans, and other aquatic insects to exemplify how river dynamics can influence the direction of gene flow and dispersal patterns of individuals, besides showing the main approaches used in this study area. By contributing to the understanding of this necessary field, we hope to stimulate new researchers to engage in the conservation of aquatic insects.

Keywords Conservation genetics · Endangered arthropods · Entomology · Habitat fragmentation · Molecular markers

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16.1 An Overview

Globally environments are disappearing faster than we can access their diversity. Scientists, environmentalists, and governments have issued dire warnings about biodiversity loss and the consequences of such losses for human health and environment permanence. There are many causes for biodiversity loss ranging from the exploitation of natural resources to the introduction of exotic species and global warming. According to World Wide Fund for Nature (WWF), a rich biodiversity is fundamental for the balance and stability of ecosystems, providing vital ecosystem services such as climate regulation, nutrient and waste management, flood control, coastal protection, provision of food and freshwater, fuel, medicines, building materials, fertile soils, and breathable air. Biodiversity has also a wide potential for economic use, especially for biotechnology. Extinction is a natural fate for any species, and occurs at some background rate; however, anthropogenic activities have increased extinction rates across species, causing catastrophic diversity loss. Preservation is imperative when it comes to aquatic environments given the intimate relationship between water and all living beings. Although aquatic environments are essential to human health, they are often the most exploited environments. Human reliance on freshwater water for consumption, agriculture and industrial purposes, and effluent dilution has resulted in freshwater vulnerability.

Recognizing the main components and dynamics of aquatic environments is the first step to establish conservation strategies. For instance, the spatial arrangement of freshwater systems is widely recognized as a fundamental attribute to be investigated. Freshwater systems are organized in drainage basins, which have distinct geological processes of formation, being composed of interconnected springs, streams, rivers, and lakes along the basin. The water direction and speed are determined by the region's topography, which can create rapids or standing water sites. The basin's arrangement is known as dendritic network, since the interconnection among streams and rivers resembles the design of a tree, with numerous small branches interconnecting and increasing in thickness as they approach to the trunk. When an ecological disturbance occurs, the direction of water flow and the disturbance origin can be used to predict negative impacts along the basins. This information combined with the regional topography can be useful to define conservation strategies within threatened areas.

Other aspects of river basins can help us to predict species diversity. For example, springs and small streams have small volumes of water and low sunlight incidence. As streams interconnect, becoming large rivers, their both the water volume and the sunlight incidence increase. For aquatic insects, the sunlight incidence and structural changes of rivers, as well as the properties of terrestrial surroundings (that may provide microhabitats), are fundamental to determine what species may occur at a given point of the watershed.

Many aquatic insects may increase or decrease in abundance in response to environmental disturbances; these species are known as bioindicators and may be used to evaluate the environmental quality of aquatic ecosystems. Changes in the relative abundance of bioindicator species may be enough to indicate whether a given environment is under stress. Thus, it is imperative to preserve bioindicators as they can alert us before environmental impacts become large enough to extinguish ecologically important species. Unfortunately, bioindicators cannot be used as universal conservation tools as they are an indirect and relatively subjective measuring of environmental quality. So what other approaches can be used to conserve biodiversity? Currently, there are several conservation approaches for aquatic organisms, but the most common practices have serious economical and logistical issues. For instance, protecting regions where threatened populations live is essential, but it is not possible for all populations. Research species conservation programs cost a lot of time and resources, and many species are widely distributed, making it infeasible to be applied for all known threatened populations. Since we are not able to protect all species at the same time, priorities should be established: How do we evaluate which species deserves more attention?

To best determine which species require the most attention, many scientists have turned to genetic tools to identify genetically diverse populations and their relative genetic connectivity. Such studies are fundamental for conservation genetics, that uses genetic analyses for management and conservation purposes. The International Union for Conservation of Nature (IUCN) recommends the preservation of high genetic diversity in populations. High genetic diversity may allow populations to more quickly adapt to environmental changes and reduce their levels of inbreeding—these two features are related to the increase in survival and fitness of natural populations. Thus, high genetic diversity and connectivity among populations—which increase genetic diversity—are one of the most important factors for the preservation of natural populations.

The importance of genetic diversity in the maintenance of natural populations has become more widely studied recently thanks to an increase in the number of conservation genetic studies that seek to combine genetic studies of populations with conservation. Many of these studies focus on understanding the mechanisms that lead to the persistence of a population and how populations respond under some natural or anthropogenic environmental stress. In this sense, this chapter provides some basic concepts to understand the studies that approach genetic connectivity in freshwater environments, focusing mainly on aquatic insects.

16.2 What Is Genetic Connectivity?

Genetic connectivity is the exchange of genetic material among populations of the same species, allowing the genetic diversity maintenance. This exchange occurs through the processes of **dispersion** and **migration**, which are movements performed by individuals or populations (Kool et al. 2013). While migration is characterized by long-distance movements, often related to seasonality—as seen in birds and fishes—dispersal is used more commonly to describe any local movements performed individuals. Dispersion can be directional when an individual goes to a "planned" place, such as its breeding site or randomly when reaching "non-planned" places, other populations, or uninhabited sites. Dispersion allows individuals to reach different populations, but genetic connectivity only occurs through mating between individuals. This reproductive connection among populations is known as **gene flow** and it may increase **genetic diversity** by introducing new alleles into a target population, changing local allele frequencies (Whitlock and Mccauley 1999).

Links among populations may be reduced or prevented due to **barriers**. Barriers are any obstacle to species dispersal. They may be structural or even environmental variations, whether naturally occurring or not. They act by separating populations although sometimes individuals may overcome barriers, resulting in the connection of two or more populations. When a barrier is strong enough, however, that no individual can transpose it, the populations become isolated, causing a genetic diversity loss (Crook et al. 2015).

The importance of gene flow for the maintenance of populations is clear, and measurements of gene flow reflect the evolutionary history of species (Ye et al. 2018). Studies on genetic connectivity have sought to understand the evolutionary processes and natural history of species. This knowledge has helped researchers to develop conservation efforts, and manage endangered species, including the mountain lion, manatee terns and harpies (Castilho et al. 2012). The pressures suffered by species vary among distinct environments, reinforcing the importance of specific studies for each species or environment: freshwater and terrestrial species greatly differ in response to environmental pressures. Within the freshwater habitat, differences occur in water flow, substrate, etc., making each environment relatively unique (Campbell Grant et al. 2007). For terrestrial species, physical structures such as vegetation, mountains, or urbanness may act as barriers to dispersal (Anderson et al. 2010). By contrast, barriers in aquatic environments are physical-chemical (i.e., temperature and water oxygen rate) more often than structural (i.e., waterfalls, dams, or rapids) (McRae 2006).

16.2.1 What Is the Importance of Genetic Connectivity in Aquatic Environments?

In freshwater systems, water speed and depth, and chemistry can act as barriers to organismal dispersion, as well terrestrial environments, that ultimately limit and shape aquatic systems (Phillipsen and Lytle 2013). Barriers may limit movements of organisms among rivers, generating high genetic structuring by isolating certain populations. Connectivity in aquatic systems has four dimensions: (1) longitudinal, throughout the channel; (2) lateral, in floodplain inundation events; (3) vertical, between the water mirror and the other water layers in environments with great depth; and (4) temporal, due to seasonal and stochastic events. Given this, McGlashan and Hughes (2001) suggest three possible scenarios: (1) negligible gene flow among drains over long periods, resulting in strong genetic structuring; (2) moderate gene flow among closely connected adjacent drains; and (3) extensive genetic flow, which reflects a high dispersion potential that can overcome the isolation imposed by drainage structures.

The structure of most basins has a tree pattern, where their trunk and branches form a structure known as a **dendritic network** (Campbell Grant et al. 2007). This structure can be found in many freshwater environments—except in isolated lakes—and is organized hierarchically, from the source (upstream) to the mouth (downstream), following the direction of water flow. This dendritic hierarchy was proposed by Robin L. Vannote et al. (1980), in a theory known as the "River Continuum Concept" (RCC). In the RCC, there are three groups of fluvial gradients, classified by its number of streams confluence: headwaters (stream order 1–3), midreaches (stream order 4–6), and lower reaches (stream order > 6) (Fig. 16.1). Each stream has different characteristics—i.e., variation in shading provided by riparian forests, sediment type, and water volume—that allow us to predict which organisms are expected to occur at each stream point. Since the environmental requirements of organisms vary along a fluvial gradient, RCC theory can be used to predict potential barriers to dispersion. This idea was tested by Meffe and Vrijenhoek (1988) and Hughes et al. (2009); they proposed a model based on RCC to explain the connectivity of aquatic populations called the "Stream Hierarchy Model" (SHM).

Under the SHM, connectivity patterns among populations of riverine species are a consequence of the dendritic arrangement of streams (Hughes et al. 2013). Under this model, populations from distinct sites within the same stream would show higher connectivity than populations present in different streams, but in the same sub-catchment. Subsequently, population connectivity would decrease among distinct sub-catchments and catchments, following a hierarchical dendritic model,

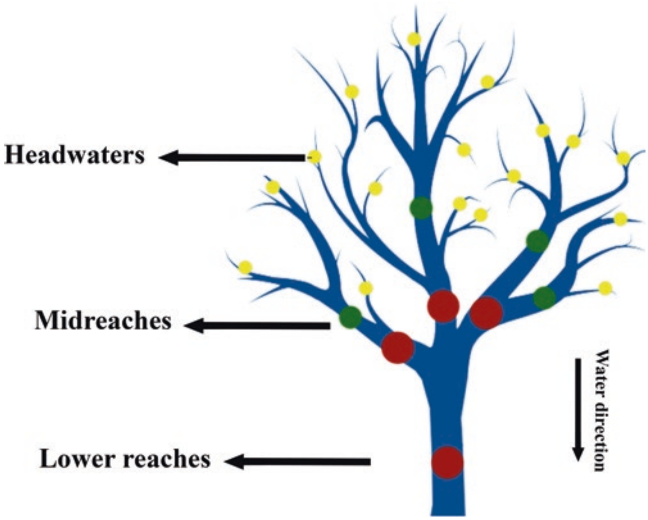

Fig. 16.1 Representation of a watershed in its tree-like dendritic pattern. The hierarchical structure is represented by circles: yellow (headwaters), green (midreaches), and red (lower reaches)

where connectivity decreases as the scales are increased. For instance, shredders—organisms that feed on coarse particulate organic material—are expected to be found in headwaters. These animals are represented by organisms such as mayflies (Ephemeroptera) or stoneflies (Plecoptera) who will feed on fallen leaves, abundant in headwaters and small streams due to riparian vegetation. In bigger streams and rivers, food might not be accessible for these organisms since most organic matter is composed of fine particles that may be taken away by strong water streams (Horne and Goldman 1994). Therefore, as RCC predicts a tree pattern, with habitats of these mayflies and stoneflies connected by large rivers, SHM predict that structural and chemical changes which occur along stream orders will be barriers to the dispersal of aquatic species, rather than the distance or isolation of streams (Keller et al. 2012).

While in lotic systems the main barriers to species distribution are the terrestrial environment between headwaters, waterfalls, and dams; the major barrier in lentic systems is the stratification of water bodies that may occur in deep lakes. Stratification is a phenomenon where different water layers of varying temperatures occur simultaneously, and the less dense layers—those with the highest temperatures—float on the denser ones with a minimum degree of mixing between them. This occurs due to the absence of aquatic currents that mix the surface water (heated by the atmosphere) with the deep water. However, stratification is a dynamic phenomenon and the water layers behave differently every season. In temperate lakes, in the early spring, when temperatures are increasing, the ice layer breaks and the water temperature increases to 4 °C, reducing its density. As summer approaches, air temperature heats surface waters and decreases their density, but deeper water remains cold due to low light incidence. In summer, there is a water layer called **thermocline**, a band of rapid temperature changes, which occurs between **hypolimnion**, the lowest and coldest lake layer, and **epilimnion**, the upper warm layer. During the fall season, the epilimnion and hypolimnion waters start to mix as their temperature equalizes until the winter, where occurs an inverse stratification, with the high-density water on top (Horne and Goldman 1994). These changes directly affect the biota in these environments. Changes in the water temperatures across seasons, for example, limits the occurrence of some species. Changes in this cycle can cause drastic population reductions (bottlenecks), that may result in a loss of genetic diversity and extinction events.

Knowing the main limitations to the dispersion of organisms is fundamental to assess the connectivity between populations. Factors such as mobility capacity, life cycle, environmental dependences, and sensibility can influence the dispersal potential of species and it should be taken into account when planning studies to assess connectivity between populations. There are several techniques for assessing connectivity between populations. However, it is always indicated to combine two or more techniques to increase the accuracy of the results and to decide what techniques are the best will depend on the organism in question. The following is a summary of the two major categories of connectivity assessment techniques seen in most studies.

16.2.2 How Can One Evaluate Connectivity?

There are several methods for estimating connectivity between populations. Since each method has advantages and disadvantages, researchers should choose methods tailored to the organisms and environments to be evaluated. Such methods are grouped into two categories: **direct** and **indirect estimates** (Kool et al. 2013). In direct estimates, individuals or gametes are samples used to estimate gene flow (i.e. Villella et al. 2004). Mark-recapture techniques are common direct approaches (Fig. 16.2); however, they are relatively imprecise about the actual gene exchange between populations (Kool et al. 2013). For example, dispersing individuals may not be successful in finding a mate if they eventually arrive at inadequate habitats that reduce their chances of a successful mating. On the other hand, indirect estimates investigate the allele frequencies between populations. They are commonly used due to its instant response, without the uncertainty of recapture and the need of waiting for individuals to travel between populations. Despite these advantages, indirect measurements have disadvantages: genetic samples assess the outcomes of past connectivity, but recent changes to populations may not be yet detectable (Rosa 2009).

16.2.3 Molecular Markers: What Genetic Analyses Are Used to Evaluate Population Connectivity?

16.2.3.1 Mitochondrial DNA (mDNA)

Mitochondrial genes are maternally inherited by the offspring. The main characteristic of mtDNA markers is their non-Mendelian pattern of inheritance, with low rate of genetic recombination. Polymerase chain reaction (PCR) can be used to

Fig. 16.2 Mark-recapture techniques are used to assess individual's movements among populations. The picture shows an individual from *Erythemis credula* (Libellulidae) marked on its right hindwing

amplify copies of the mtDNA which can be sequenced and analyzed. mtDNA are useful markers to estimate phylogenetic relationships, estimate genetic distance, and discriminate subpopulations and overall biogeographic history.

16.2.3.2 Microsatellites

Microsatellites, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs), are small sequences of DNA (1–6 base pairs), organized in tandem, randomly distributed in the eukaryotic genome. Using a small sample of DNA, microsatellites can be amplified by primers—segments of nucleic acids necessary for the initiation of DNA replication—in a polymerase chain reaction (PCR) technique, where a small and specific region of the genome is amplified by DNA polymerase enzymes. Due to their high frequency, polymorphism, and distribution along the genome of many species, microsatellites are used for tests on recent genetic splits among populations as estimators of genetic distances, discriminating subpopulations, genetic diversity, gene flow, bottleneck effects, hybridism, and individual identification.

16.2.3.3 Single-Nucleotide Polymorphism (SNP)

SNPs reflect the variation in DNA sequences at a single nucleotide in a specific position of the genome. They present low rates of mutation, and are commonly used as genetic markers for population-based, phylogeographic, and phylogenetic studies.

Freshwater Environments

Freshwater environments encompass a wide variety of formations, with different geological history, and biotic and abiotic components. They are divided into three types: lotic, lentic, and wetlands, which are a specific type of lentic system.

Lotic systems have flowing waters and can vary from springs, of few centimeters of deep, to large water bodies such as the Brazilian Amazon River, the biggest river on earth, with 6.992 km in length. Lotic systems can vary in speed, form, and size. Springs and rivers are examples of lotic systems, as are streams, creeks, waterfalls, and rapids. Rivers have unidirectional flow, continuous physical change, high degree of spatial and temporal heterogeneity at all scales, and high habitat variability throughout their orders and bear a specialized biota adapted to live in running water conditions.

Lentic systems are those where the water flows slowly or is completely stopped. They are lakes, lagoons, water puddles, wetlands, and even seasonal pools. They can range from a temporary rainwater pool with few centimeters depth to areas such as the Baikal lake of Russia, with a maximum depth of

1740 m. While lotic systems present a continuous flow that homogenizes the water temperature, lentic systems have a thermal stratification that results in water layers of different temperatures.

Wetlands are areas where the soil is saturated or flooded (permanently or seasonally). The main wetland types are swamps, marshes, bogs, fens, and peatlands. Wetlands occur on every continent and are important to water purification, flood control, and carbon sink. The largest wetlands are the Amazon River basin, the South American Pantanal, the West Siberian Plain, and the Sundarbans in the Ganges-Brahmaputra delta (Fig. 16.3).

Fig. 16.3 An example of lotic system, a stream surrounded by a riparian forest in the Cerrado biome, southeastern, Brazil

16.3 New Approaches in Freshwater Connectivity: Riverscape Genetics

There are many studies aiming to assess how habitat heterogeneity influences genetic structure of populations in terrestrial environments by combining methods from landscape ecology, spatial statistics, and population genetics (Tischendorf and Fahrig, 2000; Alp et al. 2012; Phillipsen et al. 2015). These studies comprise a sub-discipline known as landscape genetics that, more recently, has been expanded to be used in marine environments and defined as "seascape genetics" (Davis et al. 2018). Both landscape and seascape genetics try to explain how specified habitats can increase or decrease the movements of individuals and test whether certain models of individual movements can explain connectivity better than other models.

For river environments, an approach called riverscape genetics (RG) is commonly used. RG combines methods from landscape ecology and population genetics. It seeks to understand processes and factors that interfere in population dynamics to gather information that can be used to develop conservation strategies. Davis et al. (2018) define RG as “an area of study that evaluates the effect of riverscape features on spatial genetic variation.” Thus, it evaluates the effect of riverscape features on spatial genetic variation considering the riverscape as a continuum where habitats have permeable boundaries but also discontinuities among patches that can influence river organisms.

Studies in RG use continuous and discrete sampling designs to identify physical barriers and correlations between spatial genetic variation and abiotic factors. Most studies about RG, however, have been focused on discrete barriers such as waterfalls and dams (isolation by barrier hypothesis, i.e. Coleman et al. 2018) and on the physical distance among populations (isolation by distance hypothesis, i.e. Finn et al. 2006). For insects, recent studies concluded that the greatest obstacle to the dispersion of most aquatic insects is distance (Sabando et al. 2011; Short and Caterino 2009; Watanabe et al. 2008, 2010). Although their winged form can overcome most physical barriers, they often fail to travel long distances (Pfeiler and Markow 2017), with the exception of long-distance migrants like the dragonfly *Pantala flavescens* (e.g., Hobson et al. 2012; May 2013). An example of low dispersal is found in the study carried out by Chaput-Bardy et al. (2008) with the damselfly *Calopteryx splendens* (Fig. 16.4), where they found clear isolation by distance among populations. Adults cannot disperse long distances due to their limited flight capacity. By contrast, larvae can disperse greater distances than adults—despite their lower active dispersal capacity—as they are carried by the stream flow.

Fig. 16.4 The damselfly *Calopteryx splendens* (male) defending its territory on water. Photo by Kilodk on pixabay.com

Compiling several studies on riverscape genetics, Davis et al. (2018) evaluated how they have approached riverscape features in distinct aquatic taxa and pointed a few prospects for future studies. They found that studies addressing the effects of natural and anthropogenic barriers commonly seek explanations based on isolation by distance and by barriers to explain genetic diversity, but rarely these two hypotheses alone have fully explained the patterns found. Although their importance is recognized as structuring processes of genetic diversity, there is another kind of isolation that has been used to study certain organisms such as fishes. Isolation by resistance occurs when effects of multiple landscape variables, such as precipitation and temperature, are the main limitations to gene flow among populations. In this scenario, populations can respond to physical-chemical variations as barriers to dispersion more than physical barriers or distance (McRae 2006). Although there are few studies that combine all these approaches (barriers, distance, and resistance), the use of isolation by resistance concept combined with isolation by distance and barriers provides better responses to river systems.

Besides the hypotheses that try to explain connectivity patterns based on potential dispersal limits, there are others that predict the direction of gene flow. Created by Paz-Vinas et al. (2015), the Downstream Increase in Intraspecific Genetic Diversity (DIGD) hypothesis describes the asymmetrical genetic pattern found in many groups of aquatic organisms, predicting gene flow direction and estimating the genetic variation source found in a species population. DIGD suggests that dendritic networks allow asymmetrical gene flow, with the direction of gene exchange between populations usually following the water flow direction, since many species cannot travel against it (Keller et al. 2012).

Certain phenotypes can be selected by specific pressures on populations. When this occurs, organisms usually show strict adaptations that give them advantages to explore local resources better than nonspecialized neighbors, increasing their fitness. Whitehead et al. (2011) observed that living populations of the killifish *Fundulus beteroclitus* had adaptations that allowed them to tolerate pollution. In a stressed environment, these adaptations were mediated by genes related to osmotic shock, by increasing velocity response to these conditions. The link between spatial adaptive genetic variation and riverscape heterogeneity is the focus of gene-environment association (GEA) studies, which aim to find associations between genes and environment conditions.

Although GEA studies have been reporting strong correlations between genes and environment, their main challenge is in the study of wild populations. However, there are problems in assessing adaptations in wild populations regarding the ability of isolating the environmental variables of interest. An alternative to these problems is computer programs that carry out genetic selection simulations, such as LOSITAN (Looking for Selection in a Tangled dataset) (Antao et al. 2008). These programs help to understand adaptive evolution in natural populations by evaluating how dispersal and connectivity affect adaptive genes across populations. This information can be used to elucidate how environmental disturbances (such as biological invasions, diseases) affect natural populations.

An important issue is that GEA studies do not always reflect recent conditions and events. Adaptations settle in populations only after several generations, demanding a large timescale for some species depending on the time between generations. Thus, in many cases, spatial genetic structure of populations can be attributed to ancient historical events, as the Pleistocene glacial cycles or mega-flood events that affected the genetic connectivity among populations (Wong et al. 2004). Geological processes can structure or interrupt connections between populations on an entire river network. Current and past geological processes that affect genetic variation of populations allow us to preserve different species by providing information about the resilience, adaptive capacity, and natural history of populations. To evaluate the importance of past processes, scientists have developed molecular techniques capable of detecting the effects of ancient geological processes on current genetic patterns. Ye et al. (2018) using mDNA and other molecular markers found that the current connectivity patterns in *Metrocoris sichuanensis* (Hemiptera: Gerridae) were established in the last glacial event and did not change until the present. Despite the interesting results of these techniques, most studies have been conducted using fishes, which limits the extrapolation of these results to other aquatic organisms.

Riverscape genetics is a new and specialized study area that uses landscape genetics applied in river studies, but just as every new approach the methods involved are constantly being discussed and improved. Perhaps, the greatest challenge of RG today is to find a way to incorporate the dendritic spatial arrangement of rivers and the water directional movement on its modeling. Although landscape genetics addresses spatial variables, the dendritic arrangement of rivers induces organisms to exhibit dispersion patterns and connectivity that are dependent on the conformation of water bodies, flow, and physicochemical factors, and approaches that focus on these particularities are needed (Eros and Campbell Grant 2015). These particularities have been studied in seascape genetics studies; however, studies focusing on riverine organisms and other organisms than fishes can help us to establish ecological generalizations.

16.4 Genetic Connectivity in the Study of Aquatic Insects

In aquatic environments, there are several ways to use the dispersive potential of a species to approach connectivity, but knowledge regarding behavior and natural history of the involved species is required to evaluate their dispersal ability (Hughes 2007). For instance, it is known that flight is the major dispersal mechanism of winged insects while larvae and species with fully aquatic adult forms apparently move very little within water streams, resulting in significant genetic differentiation. Despite their limited dispersion, aquatic insects can eventually disperse overland. Boersma and Lytle (2014) reported one of these few events in the water bug *Abedus herberti* (Hemiptera: Belostomatidae), a flightless aquatic insect. They argue that even strictly aquatic insects, can have some level of genetic connectivity, as in drought periods in which these insects are compel to disperse overland. Differently, winged insects can travel through barriers, and some species can travel for distances as long as entire countries.

Differences in flight potential are not necessarily related among taxonomic orders. Among Odonata, for instance, there is a clear difference between the flight capability of anisopterans and zygopterans, where anisopterans, with larger bodies and wings, can fly greater distances than zygopterans, overall. Anderson (2009) and Watts et al. (2004) studied two extreme examples of dispersal ability in these groups. Anderson (2009) used mark-recapture techniques to confirm earlier reports of large-scale transoceanic dispersion in *Pantala flavescens* (Anisoptera), suggesting an incredible flight capability for this dragonfly. Conversely, Watts et al. (2004) used microsatellite markers to study the UK populations of the endangered damselfly *Coenagrion mercuriale* (Zygoptera) and found a fine-scale dispersal with high genetic structure among them, where isolation by distance develops within 10 km. While *P. flavescens* is genetically homogeneous among the continental scales due to its increased flight capacity, *C. mercuriale* has a low dispersive potential compared to other Odonata, even other Zygoptera, due to its lower flight capability and vulnerability to habitat fragmentation. This vulnerability to habitat fragmentation makes its dispersive potential relatively short, not exceeding 1.5–2 km. In addition to the high isolation by distance (IBD) (within 10 km) experienced by *C. mercuriale*, fragmentation effects make its populations more isolated than in natural conditions, increasing the risk of extinction.

Fragmentation effects harm aquatic insects as they can use the terrestrial environments as habitats, mating places, and/or corridors for migration, which allows the maintenance of gene flow among populations or subpopulations. Deforestation and land use negatively affect genetic diversity by creating barriers to dispersal and excluding adult habitats. There is a variety of studies addressing these questions, mostly with headwater-specialized insects. Alexander et al. (2011) studied the mayfly *Ephemerella invaria* (Ephemeroptera) in forested and partially deforested headwater catchments in the Mid-Atlantic Region of the Eastern United States. Using molecular markers, they found a strong negative correlation between genetic diversity and deforestation on first-order catchments. As the deforestation rate increases the genetic diversity of *E. invaria* populations decreases, so intact terrestrial environments are necessary to maintain the connectivity among populations that otherwise would be extinct. Extinction events can be followed by recolonizing events performed by survivors, but even so they may cause genetic diversity reduction by excluding populations that could be a source of genetic variation.

Isolation by distance is an important hypothesis to explain the genetic structuring in many aquatic insects as black flies, damselflies, dragonflies, caddisflies, and mayflies. Studying the Japanese caddisfly *Stenopsyche marmorata* (Trichoptera), Yaegashi et al. (2014) combined microsatellite techniques with empirical field observations of flight behavior and spatial distribution and found a significant IBD in populations separated from each other for more than 33 km. This caddisfly generally disperses along the water flow, probably occurring in both larval and adult stages, but occasionally winged adults can disperse among water catchments.

Interestingly, these researchers observed that the lateral dispersion of *S. marmorata* allows the existence of two distinct lineages, separated in upland (regions upper 250 m a.s.l.) and lowland (< 250 m a.s.l.) lineages. The main difference between them is a voltinism (number of generations per year) adaptation mediated by temperature (as we saw on GEA). Upland lineages experience monthly accumulated temperature (MAT) < 90 °C and exhibit univoltine life cycle (one reproductive event per year), whereas lowland lineages experience MAT > 90 °C and bivoltine life cycle (two reproductive events per year). Thus, besides IBD, these differences in life cycles may reduce the gene flow between *S. marmorata* populations, acting as a reproductive barrier.

Strictly aquatic insects are frequently considered to exhibit high isolation among populations from different streams, considering their low capacity of terrestrial dispersion, but that does not always occur. Miller et al. (2002) studied the dispersive potential and gene flow of *Ambrysus thermarum* (Hemiptera: Naucoridae), which has an exclusive aquatic life cycle. To evaluate its dispersive potential, they positioned traps throughout the stream margins to capture adults during dispersal movements between populations, but they did not find any of them. Hence, they did not expect to find genetic any connectivity among populations, but results showed strong genetic similarity among them. Combining these two results and the fact that seasonal streams were common in the study area, the authors concluded that there are consecutive periods of extinction and recolonization in this ephemeral environment, homogenizing the allele frequencies among populations. When there are consecutive extinctions, the new populations only have as source of genetic variation the few individuals that eventually travel between these populations, explaining the low genetic differentiation observed.

Although Miller's findings concerning *A. thermarum* emerged from recent historical events of seasonal streams, there are cases where the natural factors influencing populations are depicted from much older periods. Using mDNA, Baker et al. (2003) investigated the spatial genetic structure of the caddisfly *Cheumatopsyche* sp. (Trichoptera) in Australian streams. They suggest that *Cheumatopsyche* was isolated in small populations during the Pleistocene, but over time there was an expansion of these populations that increased the dispersion and gene flow between them. The gene flow increase caused the high genetic homogenization observed nowadays in a large geographic scale despite distance between them. Another example is the study carried out by Finn et al. (2007) with the giant water bug *Abedus herberti* in Sky Island, USA. Using mDNA as well, they found that *A. herberti* populations are isolated due to the Pleistocene climatic cycles that suppressed the connections among headwater populations. In addition, the increasing rate of climatic warming in this region accelerated the disappearance of ephemeral streams in which this species lives.

Here, we exposed a few examples illustrating how most researchers approach genetic connectivity to study aquatic insects. Hypotheses as IBD have been used to explain dispersion limitation for several taxa. However, as we have seen, the genetic connectivity patterns of each taxon will depend on life history traits and historical events that affect each environment. In addition to these factors, considering historical processes and the health of adjacent habitats is an effective way to investigate connectivity of aquatic insects.

16.5 Perspectives for the Conservation of Aquatic Insects

The importance of aquatic insects for the maintenance of aquatic and terrestrial systems has been defended for a long time. Aquatic insects contribute to many ecological functions and can admit distinct trophic positions, being filterers, collectors, predators, and others. The disappearance of one of these categories unbalances the whole system, reducing the amount of available oxygen in water, as well as affecting the rate of energy transfer. Aquatic insects transfer energy when predated by fishes and other predators, and the energy flow can even travel from the aquatic system to the terrestrial environment benefiting terrestrial predators such as birds, mammalians, and amphibians. For the maintenance of aquatic insects and their ecological functions, several conservation approaches have been developed, aiming most of the time specific species or environments. As we have seen in this chapter, genetic strategies allied with conservation strategies are helping to clarify connectivity patterns of aquatic insects and raised hypotheses that seek to explain their distribution (Frankham et al. 2012). Some hypotheses can be applied to all aquatic insects, such as IBD, and others can vary according to the environment type and the developmental stage of individuals. Even so, it is time to use this knowledge to guide management policies of aquatic and adjacent environments.

Habitat fragmentation, whether occurring directly in the aquatic environment through dams or in the terrestrial environment through deforestation, is the greatest challenge for aquatic insect conservation (Bunn and Arthington 2002). Studies performed by Fagan (2012), Petersen et al. (2004), Alexander et al. (2011), and others showed evidences of habitat fragmentation effects on genetic structure of aquatic insect populations. Even highly dispersive species may encounter barriers to their dispersion, but undoubtedly the most vulnerable species are those with limited dispersive potential. Some of them form naturally isolated populations, which makes them more susceptible to local extinctions. *C. mercuriale*, a threatened damselfly, can disperse only 10 km, but in a continuous open agricultural land its dispersal is reduced to 1.5–2 km. This scenario is not different for *A. herberti*, a naucorid, where populations are highly isolated by the headwater shortening caused by global warming. Climate change would dry completely the mountain headwaters in which *A. herberti* inhabits, causing not only its local extinction and genetic diversity loss but also of many other aquatic species. These studies may serve as a benchmark to define conservation units and should be considered when the implementation of ecological corridors (they increase connectivity among populations) and environmental restorations are necessary.

A novel approach for conserving aquatic insects is to use certain threatened species with a wide distribution as "umbrella species." It is common for many governments and society in general to not devote much attention to insect conservation, focusing attention on more charismatic groups such as mammals and birds. However, when we focus on aquatic insects it is possible to relate the preservation and health of these organisms to the quality of water and thus to human health. The main taxa associated to this idea are Ephemeroptera, Plecoptera, and Trichoptera (known as EPT); they are used in many rapid protocols that assess the quality of aquatic environments (Elbrecht et al. 2015). They inhabit headwaters and other aquatic environments relatively undisturbed, and can be used to protect a wide variety of other taxa that depend not only on aquatic habitats, but also on the terrestrial ones.

To conclude, the conservation of aquatic insects directly depends on preserving their aquatic systems, which ultimately depicts the most valuable resource for us humans: the water. Nonetheless, all approaches depend on governmental managements and the creation of policies that minimize the human impacts on these systems. Studies that take into account genetic composition of populations for the establishment and maintenance of genetic diversity sources are necessary for biodiversity conservation. However, anthropogenic issues are beyond the directly observed impacts on aquatic systems. As some studies have shown, global temperature is still rising and wildlife is suffering with a fast environmental change that most species are not able to deal with. Freshwater systems are dependent on the water supply in wet seasons for their persistence; thus changes in rainfall regimes in addition to the exploitation of water tables can impact these environments at large scales, affecting the fauna and human needs. In this scenario, it is essential to optimize the natural resource conservation using modern tools, such as genetic markers allied to large-scale public policies that address the diversity loss problem as a real threat to the human health.

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