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Genetic Diversity and Structure of *Anax imperator* Leach, 1815 Populations (Odonata: Aeshnidae) in Ponds at Regional and European Scales

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Abstract: Anthropogenic activities cause loss and fragmentation of natural habitats and have strong effects on population maintenance by increasing their isolation. Pond ecosystems are scattered waterbodies that can interact as a network connected by dispersal events of freshwater organisms. Identifying local genetic differentiations and understanding how gene flow occurs across these networks is essential to prevent risks associated with environmental perturbations. This study aimed to investigate genetic diversity and structure of *Anax imperator* Leach, 1815 populations at both regional and European scales using seven microsatellites markers. Seven populations of *A. imperator* were sampled in northwestern France and four populations were sampled in Italy (Sicily), Czech Republic, Switzerland and United Kingdom (U.K.). French populations presented a low genetic differentiation indicating a high gene flow and confirming dispersal events of this species between ponds at regional scale. No pattern of isolation by distance was found at the European scale. The populations presented a low genetic differentiation and no pattern of isolation by distance, suggesting historical or current movements of individuals. Only the U.K. population presented a significant genetic differentiation from other European populations, suggesting that the English Channel might act as a barrier to gene flow for *A. imperator*. However, Bayesian analysis showed that some dispersal events could occur between the U.K. and France (Normandy), probably facilitated by prevailing winds.

Keywords: dispersal barriers; dragonflies; genetic differentiation; pond networks; population structure

Citation: Minot, M.; Husté, A. Genetic Diversity and Structure of *Anax imperator* Leach, 1815 Populations (Odonata: Aeshnidae) in Ponds at Regional and European Scales. *Diversity* **2022**, *14*, 68. https://doi.org/10.3390/d14020068

Academic Editors: Michael Wink, M. Olalla Lorenzo-Carballa and Ricardo Koroiva

Received: 16 December 2021
Accepted: 16 January 2022
Published: 20 January 2022

1. Introduction

The spatial structure of populations is generally conditioned by intrinsic life traits (e.g., dispersal capacities), distances between sites and environmental factors such as physical barriers or climate gradients [1–3]. Anthropogenic activities modify landscape characteristics leading to loss and fragmentation of natural habitats [4]. Consequently, many wildlife populations live in isolated habitat patches and often suffer a loss of genetic diversity due to inbreeding [5,6]. Genetic studies are crucial to drive species conservation measures because a low genetic diversity also increases the risk of population extinction due to environmental perturbations and demographic stochasticity [7]. The local genetic diversity can vary independently from the geographical distribution of the considered species. Some wide-ranging species with high dispersal capacities can have different genetic diversities at smaller scales [8], while other ones with low dispersal ability can have low genetic differentiation at larger spatial scales [9,10]. In rare species, the genetic diversity can also be constraint by specific habitat requirements that often induce isolation of populations [11,12]. Overall, the genetic diversity depends on gene flow that is conditioned by the frequency of dispersal events between populations [13].

Most dispersal events cover only short distances (i.e., short distance dispersal; SDD) and take place within the boundaries of defined geographic or population limits [14].

However, these SDD events may also allow distant populations to connect by a 'step by step' dispersal process [15,16]. On the contrary, long distance dispersal (LDD) movements are generally rare and difficult to detect [17,18], except in migratory species [19,20]. The LDD often involve physical forces like wind and marine currents [21,22] or rely on organisms with higher dispersal abilities [16,23]. Dispersal events have major consequences on population dynamics. They enable species to colonize new patches and expand the occupancy on their territory. They also allow the maintenance of population dynamics in patches where species are already present and genetic mixing with other populations [14]. Both SDD and LDD are crucial for population maintenance because they limit the risk of persistent extinction on one site by spreading local temporal dynamics of extinction and colonization on multiple sites. Moreover, they preserve genetic diversity [13] which increases the resilience of populations to environmental changes (e.g., climate change, invasive species, habitat fragmentation) [24,25]. Estimating dispersal is often difficult in the field because these events are rare at the individual scale. However, genetic techniques can provide accurate estimates of gene flow between populations and therefore indirect measurements of dispersal [26,27].

Ponds are small waterbodies with high conservation interest because of their high biodiversity of aquatic plants, macroinvertebrates and amphibians [28,29]. Although ponds are often scattered elements in the landscape, they are regularly considered as working in networks. Interestingly, stepping-stone models (i.e., models in which individuals can move among an infinite array of populations) [30] can be applied to these ecosystems to investigate the genetic structure of pond populations [31,32]. The persistence of populations is constrained by the availability of suitable breeding ponds, the distance between them, as well as the nature of the habitats crossed, and the dispersal capacities of the considered species [33]. Finally, pond populations can be threatened by perturbations like water pollution or summer droughts [28,34,35]. Determining how gene flow is distributed across pond networks and identifying potential local genetic differentiations is essential to assess population decline and extinction risks associated with environmental perturbations and habitat fragmentation.

Odonates are insects with aquatic nymphal development and terrestrial (aerial) adults [36]. While some rare dispersal events have been reported at the nymphal stage, the vast majority of the movements are performed by flying adults [37]. Dispersal distances are difficult to quantify and can vary a lot depending on the dispersal ability of the species. For instance, most zygopteran species do not move over more than one kilometer during their lifetime, whereas some large Anisoptera can fly over several kilometers within minutes [38,39]. In many species, most individuals stay on the same pond during their whole lifetime [40–42], whereas other species like *Pantala flavescens* Fabricius, 1798 can undertake recurrent migration flights across the oceans [43]. Therefore, the degree of genetic differentiation between odonate populations depends on dispersal traits like body size or wing morphology, but also on their behaviour and ecological niche. For zygopteran species with similar body size, a specialist species like *Coenagrion mercuriale* Charpentier, 1840 [44] shows considerable genetic differentiation between populations at a local scale (i.e., within a distance of 24 km), whereas a very weak genetic structure was found at European and North-American scales for the generalist species *Ischnura elegans* Vander Linden, 1820 [45] and *I. hastata* Say, 1839 [46], respectively. Several landscape features might also act as a physical barrier to dispersal, limiting the gene flow between populations. For instance, dispersal movements of *C. mercuriale* are hindered by small hills, or patches of trees and shrubs [47,48].

Anax imperator Leach, 1815 is a large dragonfly species (i.e., body size between 7 and 8 cm). Its distribution ranges from South Africa to Sweden [49] and seems to expand very quickly (ca. 88 km per year) in response to the global climate change [50]. No migration on long distances is reported for this species [38] contrary to other Aeshnidae like *A. junius* Drury, 1773 which is known to migrate along the Eastern coast of the USA [51]. Nymphs and exuviae measure up to 5 cm [52] and can be found sometimes in high densities in large

sun-exposed ponds with well-developed aquatic vegetation [53]. Mature adults present a territorial behaviour but are also very mobile around their mating sites. Movements of individuals have been recorded over few kilometers only, whereas this species is expected to undertake flights on much longer distances [54]. However, lack of information on long-distance dispersal events of *Anax imperator* can be explained by the difficulty to track insects during long periods with available capture-mark-recapture techniques.

The present study focused on eleven populations of *Anax imperator* from ponds in two Western European countries (i.e., France (Normandy) and United Kingdom) and three Central and Southern European countries (i.e., Switzerland, Czech Republic and Italy (Sicily)). Thus, genetic diversity and gene flow were investigated at both the regional (i.e., populations from Normandy) and the European scales. Samples consisted either of adult legs, nymphal legs, fresh exuviae (i.e., collected within the 24 h after emergence) or old exuviae (i.e., collected at an unknown date after ecdysis). Therefore, the DNA exploitability between fresh and old exuviae was also compared. Since *A. imperator* is a large dragonfly with high dispersal abilities between its breeding sites, only a weak genetic differentiation was expected between populations at a regional scale. Nevertheless, higher genetic differentiation was expected at the European scale. We also hypothesized that geographical barriers, and especially the English Channel or mountain chains such as the Alps, might limit gene flow between populations at the European scale.

2. Materials and Methods

2.1. Study Populations and Sample Collection

Samples were collected from seven localities in France (Normandy) and a single locality in the other countries (i.e., United Kingdom, Switzerland, Czech Republic and Italy (Sicily); Table 1. A total of 251 individuals (i.e., 6 to 39 per locality) was collected at or near ponds for DNA analysis. Depending on localities, different types of samples were collected: a hind-leg tarsus of adults, a hind-leg tibia of nymphs, fresh or old exuviae (Table 1). Some exuviae used for the analyses were obtained from individuals that were reared at the University of Rouen (Normandy, France). They were collected within the 24 h following emergence and were therefore qualified as "fresh". Other exuviae collected in the field were qualified as "old". Indeed, the date of emergence was unknown and since this exoskeleton can persist over several weeks in the vegetation [55,56], we had no idea of the delay between emergence and collection. After collection, all samples were stored in 99.5% ethanol until DNA extraction.

Table 1. Details on the 11 localities where populations of *Anax imperator* that were studied in Europe and the number of collected samples according to their source of DNA. NAs indicate no information on sex available.

Country	Pop	Site Name	Coordinates (WGS84)	Sex		Source of DNA			
				Males	Females	Adult Legs	Nymphal Legs	Fresh Exuviae	Old Exuviae
Italy	1	Sicily	37.086 N, 15.286 E	NA	NA	6			
Switzerland	2	Neuchâtel	47.002 N, 6.741 E	12	4	16			
Czech Republic	3	Kyjov	49.010 N, 17.128 E	7	21		16		12
France	4	Beaussault	49.682 N, 1.555 E	NA	NA		33		
France	5	Cerisy	49.199 N, −0.912 E	NA	NA		19		
France	6	Heudreville	49.133 N, 1.198 E	18	22			36	4
France	7	Bois-Guillaume	49.480 N, 1.102 E	25	14			39	
France	8	Marchésieux	49.178 N, −1.324 E	9	5		14		
France	9	Paluel	49.835 N, 0.624 E	8	7			15	
France	10	Bresle	49.914 N, 1.679 E	7	3				10
United Kingdom	11	York	53.964 N, −1.086 E	15	16				31
				Total (n = 251)		22	82	90	57

2.2. DNA Extraction and Microsatellite Genotyping

Collected legs were cut to smaller fragments using scissors. Then, DNA extraction was performed using QIAamp Micro kits (QIAGEN, Courtaboeuf, France), following the protocol provided with the kits. Collected exuviae were dried on a glass surface during the night before extraction to allow alcohol evaporation. The exuviae were cut with scissors to keep only the thorax, legs and the white tracheal lining from the abdomen. The material was placed in a 5 mL Eppendorf tube with three steel beads and homogeneously grinded with a MM400 mixer mill (Retsch, Éragny, FRance) for three minutes according to the protocol proposed by [57]. Finally, DNA extraction was performed using DNeasy Blood & Tissue Kits (QIAGEN, France), following the protocol provided with the kits except for the following changes: quantity of proteinase K was 25 µL, quantities of buffer AL and ethanol were 250 µL after incubation and as suggested by [57] and the elution step was performed twice with 50 µL AE buffer. Individuals were genotyped using 12 microsatellite loci previously developed for *A. imperator* [58]. Among these loci, two were derived from the sister species *A. parthenope* and showed successful amplification with *A. imperator*. Primers 5′-labelled with 3 fluorescent dyes (i.e., FAM, VIC and PET; Life Technologies SAS, Villebon-sur-Yvette, France) were used for amplification reaction in four separate PCR multiplexes in a thermocycler (Mastercycler nexus gradient Eppendorf, Montesson, France). Polymerase Chain Reactions (PCRs) were performed in total volumes of 12.5 µL containing 6.25 µL Qiagen Multiplex PCR Master Mix (QIAGEN, France), 4 µL RNAse-free water, 1.25 µL of one of the four multiplexed primer combinations (concentration of each primer: 2 µmol/µL) and 1 µL of DNA (10 ng/µL). PCR were performed using the following thermocycler program (QIAGEN): first an initial denaturation step at 95 °C for 15 min, then 35 cycles of denaturation consisting in 30 s at 93 °C, 90 s at an annealing temperature of 52 °C or 57 °C depending on the multiplexes used and an elongation at 72 °C for 60 s, and finally an extension at 65 °C for 30 min. Finally, 1 µL of each PCR product was added to a solution of 8.8 µL of formamide (Applied Biosystems, Villebon-sur-Yvette, France) and 0.2 µL of GeneScan 600 LIZ size standard (Applied Biosystems). Fragments were analysed by capillary electrophoresis using an ABI Prism 3500 Genetic Analyzer (Applied Biosystems). Then, results were analysed with the GeneMapper 4.1 software (Applied Biosystems).

2.3. Genetic Diversity

All analyses were performed using the R software [59]. Means are given ± SD.

The presence of null alleles was checked using the R package "PopGenReport" [60]. Deviation from expected Hardy–Weinberg Equilibrium (HWE) conditions for each locus and each population was tested using the R package "pegas" [61] and an exact test based on 10,000 Monte Carlo permutations of alleles. Linkage Disequilibrium (LD) between all locus-pair combinations was tested using the R package "genepop" (version 1.1.7) [62,63]. Markov chain parameters were 1000 dememorization, 100 batches and 1000 iterations per batch for each test. *p*-values in the detection of HWE and LD were corrected with a False Discovery Rate (FDR) procedure using Benjamini-Hochberg-Yekutieli method [64,65].

Observed heterozygosity (Ho), expected heterozygosity (He), numbers of alleles (Na), allelic richness (AR), and inbreeding coefficients (FIS) for each population were calculated using the R package "diveRsity" (version 1.9.90) [66]. The AR was calculated using the rarefaction method to correct for variation in sample size [67] and to avoid having to exclude a population from analyses. The 95% confidence intervals (CI) for FIS estimates were calculated using 10,000 bootstrap iterations.

The global measures of FIS and FST, as well as pairwise FST-values between all populations, were calculated using the diveRsity package. FST is considered as an effective measure for population genetic differentiation when using relatively small data sets with fewer than 20 loci [68,69]. All these F-statistics used the bias-corrected formulation of Weir and Cockerham [70]. Estimate 95% confidence intervals for all measures of differentiation were calculated using 10,000 bootstrap iterations.

2.4. Population Genetic Analyses and Geographic Structure

We used the pegas package [61] to perform an analysis of molecular variance (AMOVA) [71] based on Euclidian distances among individuals for all microsatellite loci. The AMOVA was conducted to partition total genetic variation across three hierarchical levels: among countries (i.e., U.K., France, Switzerland, Czech Republic and Sicily), among populations within countries and within populations. The statistical significance of the fixation indexes Φ was calculated using 10,000 permutations of data.

Genetic isolation-by-distance (IBD) is defined as a decrease in a genetic similarity among populations as the geographical distance between them increases. It was investigated considering a two-dimensional stepping-stone model and by studying the correlation between FST/(1-FST) and the natural-log-transformed (ln) geographic distance [72]. A Mantel test between a matrix of genetic differentiation between *A. imperator* populations (i.e., using FST/(1-FST)) and a matrix of Euclidean distances between these populations was performed using the package "ade4" with 10,000 permutations.

To investigate the genetic structure of the 11 populations of *A. imperator* sampled, a model-based clustering was performed using the STRUCTURE 2.3.4 program [73]. It uses a Bayesian Markov chain Monte Carlo (MCMC) method to identify genetic clusters (K) and assign individuals to these clusters. Each cluster is characterised by a set of allele frequencies at each locus. Individuals are assigned to these clusters based on the likelihood of their multilocus genotypes to belong to these genetic clusters by minimising deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) [73]. We performed runs for a number of clusters (K) ranging from two to eight and with a number of 20 independent runs for each K. *Anax imperator* was expected to have high dispersal abilities leading to frequent exchanges of individuals between populations. Therefore, an admixture model with correlated allele frequencies was considered. The LOCPRIOR parameter was not considered, i.e., the geographic location of the individuals was not considered as an additional information. For each model, a burn-in period of 100,000 followed by 1,000,000 iterations was used to ensure convergence of the MCMC. The optimum number of clusters was identified using both the log-likelihood (lnP (K)) and the estimated ΔK for each K following [74]. The CLUster Matching and Permutation Program (CLUMPP) [75] was used to aggregate all STRUCTURE runs for the optimum identified value of K. STRUCTURE models, identification of K following the Evanno's method, CLUMPP analyses and visualisation of the individual Bayesian assignment probability for the optimum value of K were performed using the R package STRATAG [76].

Since French populations sampled were geographically close, we also investigated genetic structure in individuals using a Discriminant Analysis of Principal Components (DAPC) performed with the R package ADEGENET 2.1.3 [77].

Spatial genetic structure was also investigated using a spatial model in the R package GENELAND 4.9.2 [78]. Like STRUCTURE, GENELAND provides tools to identify clusters of individuals using Bayesian MCMC inferences with genetic data by maximizing Hardy–Weinberg equilibrium and minimizing linkage disequilibrium, but geographical coordinates of individuals are also considered to inform prior distribution. This spatial clustering method allows inference of the borders between inferred clusters and is a powerful method for detecting linear barriers to gene flow between populations [79]. The GENELAND analysis was performed using four independent runs and for each run, a number of clusters K ranging from Kmin = 1 to Kmax = 8 with 1,000,000 MCMC iterations, a burn-in period of 1000 and a thinning value of 100. We used a correlated allele frequency model that considered account the potential presence of null alleles. The best run was selected according to the highest average posterior probability given by GENELAND.

2.5. Migration Rates between Studied Populations

To estimate recent migration rates between populations (i.e., over the last several generations), analyses using MCMC were conducted in BAYESASS 3.0.4 software [80]. The model was first run considering default values of the mixing parameters for migration rates

(i.e., 0.1), allele frequencies (i.e., 0.1) and inbreeding coefficients (i.e., 0.1). The acceptance rates given by BAYESASS of each of these three mixing parameters must be comprised optimally between 20% and 60% [81]. Since the acceptance rates were first higher than 60%, the model used ran with higher values of the mixing parameters (i.e., 0.7 for migration rate, 0.65 for allele frequencies and 0.8 for inbreeding coefficients) to ensure that all these acceptance rates fall in the acceptable range, and a burn-in of 1 × 106 for 1 × 107 iterations.

3. Results

3.1. Genetic Diversity

Two of the 12 microsatellites considered by [58], i.e., AiK04 and AiG03, were not retained because the first was monomorphic and the second was not successfully amplified. Loci AiJ04, AiL04 and AiM04 had high frequencies of null alleles (0.25, 0.25 and 0.23, respectively) and were also not retained. Then, individuals with more than three loci with missing values were removed from the data. Ten samples from fresh exuviae were excluded (i.e., 11.1% of the total number of fresh exuviae) and 18 samples from old exuviae were excluded (i.e., 31.6% of the total number of old exuviae). No sample from adult or nymphal legs had more than three missing loci. Finally, 223 individuals were considered for further analyses on seven markers (Table 2) and the total remaining missing values represented 6.6% of the loci.

Table 2. Genetic diversity measures (mean ± SE) in the 11 sampled populations of *Anax imperator* from Europe. Legend: *n* = number of sampled individuals, H_o = observed heterozygosity, H_e = expected heterozygosity, N_a = number of alleles, A_R = allelic richness, F_{IS} = inbreeding coefficient. Bolded H_o indicate populations presenting a significant departure from HWE condition. Bolded F_{IS} indicate a bootstrapped 95% confidence interval that does not overlap zero.

Country/Site	Pop	*n*	H_o	H_e	N_a	A_R	F_{IS}
Italy (Sicily)	1	6	0.71 ± 0.07	0.62 ± 0.05	29	4.14 ± 0.46	−0.17
Switzerland	2	16	0.65 ± 0.06	0.61 ± 0.06	42	4.02 ± 0.44	−0.06
Czech Republic	3	21	0.61 ± 0.07	0.65 ± 0.06	47	4.26 ± 0.57	0.06
France (Beau.)	4	33	0.61 ± 0.07	0.65 ± 0.06	56	4.39 ± 0.52	0.06
France (Cerisy)	5	19	0.59 ± 0.08	0.68 ± 0.05	46	4.40 ± 0.54	0.15
France (Heud.)	6	32	**0.47 ± 0.06**	0.67 ± 0.06	60	4.75 ± 0.54	**0.30**
France (Bois-G.)	7	34	0.59 ± 0.06	0.69 ± 0.05	54	4.63 ± 0.45	**0.16**
France (Marc.)	8	14	0.75 ± 0.07	0.72 ± 0.04	46	4.76 ± 0.47	−0.03
France (Paluel)	9	14	0.57 ± 0.06	0.66 ± 0.05	45	4.54 ± 0.38	0.15
France (Bresle)	10	9	0.43 ± 0.08	0.61 ±0.06	31	4.05 ± 0.53	0.25
United Kingdom	11	25	**0.33 ± 0.11**	0.63 ± 0.05	37	3.96 ± 0.35	**0.51**

Globally, populations showed substantial genetic variations. Estimates of observed and expected heterozygosity were close and ranged from 0.33 to 0.75 and from 0.61 to 0.72, respectively (Table 2). Only the population from U. K. presented an observed heterozygosity (0.33) significantly smaller than the expected heterozygosity (0.63). The total number of alleles over all loci ranged from 29 alleles in the population from Italy (Sicily), where the sample size was also reduced compared to the other locations, to 60 alleles in the Cerisy population (France; Table 2). A significant positive correlation was observed between the number of sampled individuals and the total number of alleles over all loci (Pearson correlation test: r = 0.84, p = 0.0013). Estimates of allelic richness per locus were similar between populations (Table 2).

Most markers met HWE conditions in each population except in the two populations showing departure from HWE conditions (i.e., U.K. and Heudreville, $p < 0.05$; Table 2). Because these departures of markers from HWE were not systematic in all populations, all markers were retained for further analyses. All other populations met HWE conditions ($p > 0.05$). FIS values showed significant deviation from zero in the two populations that did not meet HWE, indicating homozygosity excess in these two populations, and especially in

the U.K. population (Table 2). Linkage disequilibrium (LD) tests for each pair of loci over all populations indicated no evidence for significant disequilibrium (all $p > 0.05$).

3.2. Population Genetic Differentiation

Global F_{IS} (0.1615, 95% CI = 0.1233–0.2000) and F_{ST} (0.0224, 95% CI = 0.0101–0.0366) were greater than zero. Pairwise F_{ST}-values ranged between −0.0151 and 0.1297.

A moderate genetic differentiation (i.e., 95% bootstrapped CI) was particularly found between U.K. and all other populations (all $F_{ST} > 0.081$). A moderate differentiation was also found between the Swiss and one of the French populations (i.e., Marchésieux, $F_{ST} = 0.046$; Table 3).

Table 3. F_{ST}-values between all populations. Bolded values indicate a bootstrapped 95% confidence interval that does not overlap zero. The mean F_{ST}-value is 0.0306 ± 0.0051.

	Italy (Sicily)	Switz	Cz. Rep.	France (Beaus.)	France (Cerisy)	France (Heud.)	France (Bois-G.)	France (March.)	France (Paluel)	France (Bresle)	U.K.
Italy (Sicily)	-	0.0293	0.0307	−0.0079	0.0163	0.0047	0.0104	0.0097	−0.0132	0.0254	**0.1055**
Switzerland		-	0.0128	0.0167	0.0111	0.0234	0.0192	**0.0446**	0.0126	0.0481	**0.1220**
Czech Republic			-	0.0110	0.0232	0.0272	0.0206	0.0291	0.0164	0.0509	**0.1148**
France (Beau.)				-	0.0037	0.0115	0.0143	0.0036	−0.0021	0.0191	**0.1297**
France (Cerisy)					-	−0.0059	0.0045	0.0049	−0.0063	−0.0055	**0.0903**
France (Heud.)						-	−0.0007	0.0019	−0.0031	−0.0151	**0.0843**
France (Bois-G.)							-	0.0183	0.0009	0.0193	**0.0841**
France (Marc.)								-	0.0052	0.0119	**0.0920**
France (Paluel)									-	0.0043	**0.1014**
France (Bresle)										-	0.0813
United Kingdom											-

The AMOVA analysis showed that most molecular genetic variation resulted from individual genetic variation within populations (92.73%; Table 4), the remainder (6.37%) resulting from genetic variation among countries ($p = 0.02$). Variation among populations within countries related only to French populations, since only one population was analyzed in the other countries. No significant genetic variation was found among populations sampled in France (0.90%, $p = 0.09$).

Table 4. Results of the AMOVA performed for the 11 population of *Anax imperator* sampled in the five European countries studied.

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variance	Φ-Statistics	*p*-Value
Among countries	4	138.79	0.78	6.37	$\Phi_{CT} = 0.06$	0.02
Among populations within countries	6	82.21	0.11	0.90	$\Phi_{SC} = 0.01$	0.09
Within populations	212	2405.45	11.35	92.73	-	-
Total	222	2626.45	12.24	100.00	-	-

No evidence for isolation by distance was found among populations at the European scale, since the correlation between genetic and geographic distance matrices was not significant (Mantel test: r = 0.33, $p = 0.17$; Figure 1). However, three ellipses could be visually drawn to delimitate three point clouds: the U.K. population versus all others (1), French (Normandy) populations within each other (2) and populations from Central and Southern European countries (i.e., Switzerland, Czech Republic and Italy (Sicily)) versus French (Normandy) populations (3). Although no significant differentiation was found, these point clouds show a higher genetic differentiation between U.K. populations and all other populations (i.e., ellipse 1) than between French (Normandy) populations themselves (i.e., ellipse 2) and between French (Normandy) and the three populations from Central and Southern European countries (i.e., ellipse 3).

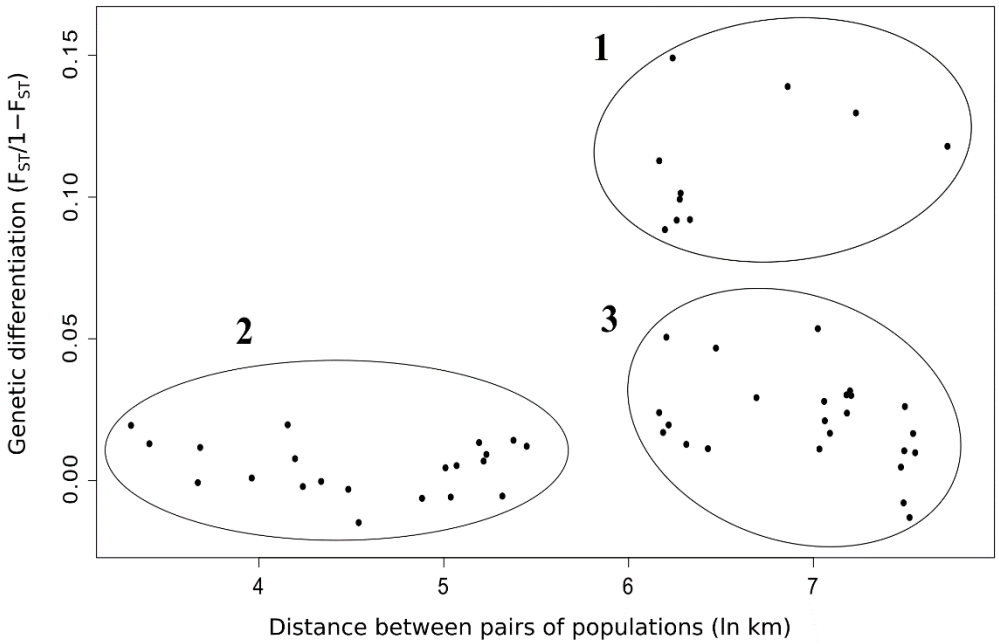

Figure 1. Relationship between pairwise population differentiation ($F_{ST}/1 \div F_{ST}$) and the geographic distance (ln km) separating populations. Ellipse 1 represents pairwise differentiations between U.K. and all other populations. Ellipse 2 represents pairwise differentiations between the French (Normandy) populations. Ellipse 3 represents pairwise differentiations between the French (Normandy) populations and the three populations from Central and Southern European countries (i.e., Switzerland, Czech Republic and Italy (Sicily) and among these three populations.

3.3. Spatial Genetic Structure

STRUCTURE analyses identified three genetic clusters since values of lnP (K) and ΔK showed a peak at K = 3 (Figure S1a,b). A first group contained populations from Switzerland, Czech Republic and Italy (Sicily) (Figure 2a,b). A second group contained the U.K. population (Figure 2a,b). A third group contained all French populations (Figure 2a,c).

DAPC on French populations only was performed retaining 70 Principal Components (PC) and two discriminant functions. It suggested three subclusters: one with the population of Bresle, a second with the population of Marchésieux and a third with the five other Normandy populations in which the population of Bois-Guillaume was slightly detached from the four other populations (Figure S2).

All independent runs performed in the spatial model given by GENELAND corroborated STRUCTURE results and identified three genetic clusters. The run with the highest average log posterior probability was retained. The MCMC converged within the 100,000 iterations. The U.K. and Bresle populations were assigned to one cluster with a probability of at least 0.7 (Figure S3a). Most of other Normandy populations except Beaussault were assigned to a second cluster with a probability of at least 0.7 (Figure S3b). Normandy population from Beaussault and populations from Switzerland, Czech Republic and Italy (Sicily) were assigned to a third cluster with a probability of at least 0.7 (Figure S3c).

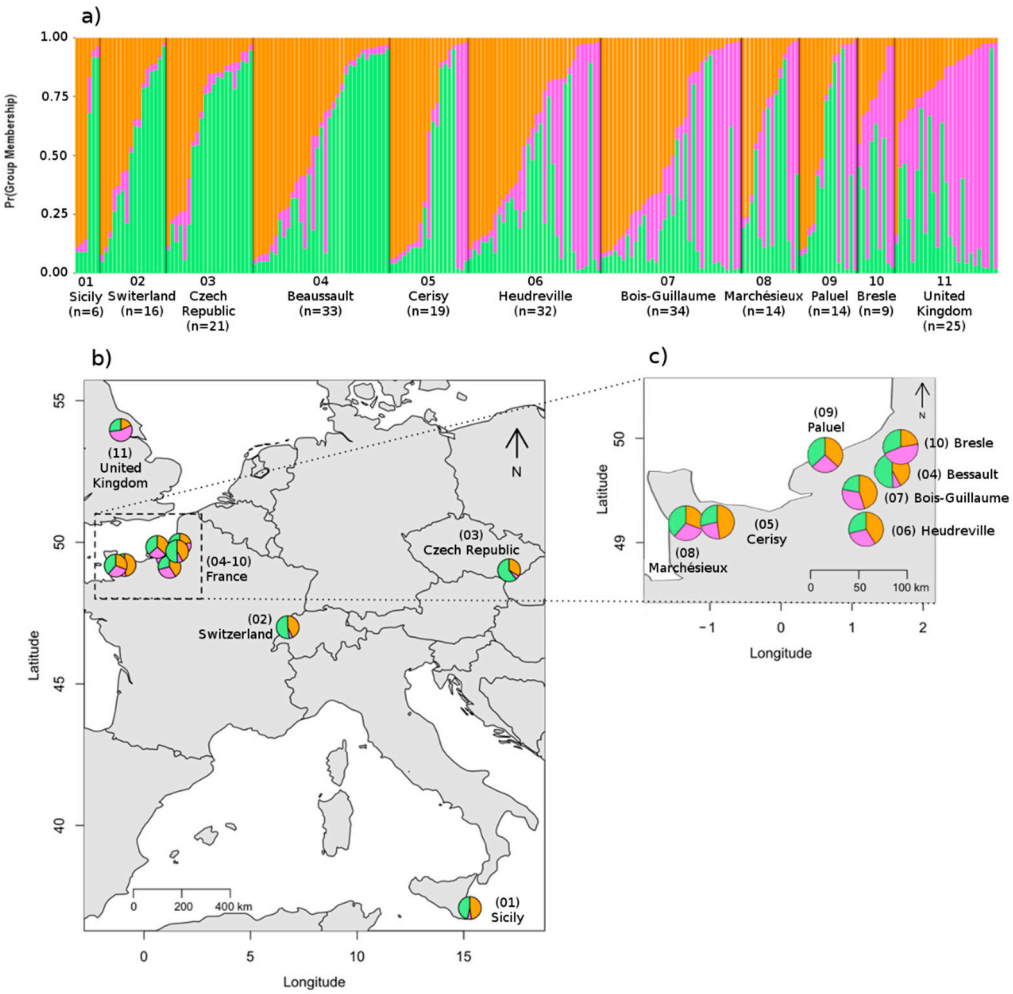

Figure 2. Results of individual assignments to each cluster by STRUCTURE. (**a**) Probabilities of individual membership in the 11 sampled populations, with bars representing individuals and colours representing the probability of belonging to the three genetic clusters identified with STRUCTURE. (**b**) Mean membership in the 11 sampled populations in Europe to each of the three clusters. French populations are delimited in a dotted rectangle. (**c**) Enlarged view of the French populations.

3.4. Recent Migration Rates among Populations

Bayesian analyses clearly showed several directional gene flows between the studied populations. The U.K. population was rather isolated from the other populations but it was a donor site only for the Bresle population from France (Table 5). All the three populations located in Central and Southern European countries (i.e., Switzerland, Czech Republic and Italy (Sicily)) showed no migratory exchange between them and were not sources for French populations. The French population from Beaussault, and especially the French population from Cerisy, were sources for Swiss, Sicilian and Czech populations (Table 5). In France especially, the population from Cerisy was identified as likely one of the main donors for the other four French populations. The populations from Heudreville and Beaussault seemed particularly implicated in source-sink processes, since they acted both as donor and target populations. The French populations from Paluel and Bois-Guillaume were identified as receivers only. The French population from Marchésieux seemed isolated from all other populations since no exchange was identified (Table 5; Figure 3).

Table 5. Bayesian modelling of potential bias in direction of dispersal (gene flow) among the 11 populations of *Anax imperator* in Europe. Numbers represent the proportion that disperses between sites (bold indicates self-recruitment). Values <0.05 (5%) are in grey. Italic indicates pairs of sites with ≥10% exchange. These values represent historical gene flow, and do not provide any information about contemporary levels of dispersal among sites.

Target	Potential Donor Site										
	Italy (Sicily)	**Switz.**	**Cz. Rep.**	**France (Beaus.)**	**France (Cerisy)**	**France (Heud.)**	**France (Bois-G.)**	**France (March.)**	**France (Paluel)**	**France (Bresle)**	**U.K.**
Italy (Sicily)	**0.68**	0.02	0.02	0.05	*0.10*	0.02	0.02	0.02	0.02	0.02	0.02
Switzerland	0.01	**0.68**	0.02	0.07	*0.12*	0.01	0.03	0.01	0.01	0.01	0.01
Czech Republic	0.01	0.02	**0.69**	0.04	*0.17*	0.01	0.02	0.01	0.01	0.01	0.01
France (Beau.)	<0.01	0.02	0.03	**0.69**	*0.18*	0.01	0.01	0.01	<0.01	<0.01	<0.01
France (Cerisy)	0.01	0.02	0.03	0.03	**0.78**	0.02	0.02	0.01	0.01	0.01	0.04
France (Heud.)	<0.01	0.01	0.01	0.03	*0.13*	**0.72**	0.04	<0.01	<0.01	<0.01	0.02
France (Bois-G.)	<0.01	0.01	0.02	0.02	*0.12*	0.06	**0.73**	<0.01	<0.01	<0.01	0.01
France (Marc.)	0.02	0.02	0.02	0.04	*0.10*	0.04	0.02	**0.69**	0.01	0.01	0.03
France (Paluel)	0.01	0.02	0.02	0.05	0.08	0.05	0.02	0.02	**0.68**	0.01	0.02
France (Bresle)	0.02	0.02	0.02	0.04	0.04	0.03	0.02	0.02	0.02	**0.68**	*0.11*
United Kingdom	<0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	<0.01	0.01	**0.87**

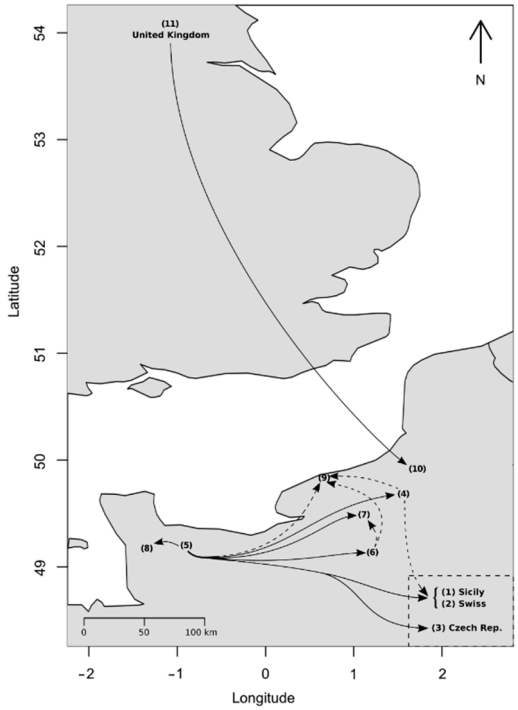

Figure 3. Map of the gene flow between the 11 populations of *Anax imperator* in Europe. Arrows indicate gene flow between pairs of sites with ≥5% exchange (dashed lines) and ≥10% exchange (solid lines). Numbers in parentheses are numbers assigned to populations in Table 1.

4. Discussion

Anax imperator populations sampled in the Normandy region in France had a low level of genetic differentiation (i.e., F_{ST}-values all below 0.03) compared to previous studies on European odonates at a local or regional scale (i.e., F_{ST} up to 0.08, 0.10, 0.24 and 0.28 for *Leucorrhinia dubia* Vander Linden 1825, *Coenagrion scitulum* Rambur 1842, *C. mercuriale*, respectively) [45,82–84]. Such results confirm the high mobility of *A. imperator* [85] and its efficient dispersal between ponds at the regional scale [54]. However, at the European scale, a moderate level of genetic differentiation was found between populations. In particular, the population sampled in the United Kingdom presented the highest genetic differentiation from the other populations sampled (i.e., F_{ST} up to 0.13). Moreover, movements of individuals seem not to occur between all populations at local and European scales, mean-

ing that all ponds may not play the same role in maintaining exchanges and a posteriori population viability.

All populations presented an observed heterozygosity close to expected levels, except the population sampled in the U.K. In this population, observed heterozygosity was lower than expected heterozygosity and allelic richness was lower compared to other populations. This lower genetic diversity was also associated with a significant degree of inbreeding. Low genetic diversity and inbreeding suggest a possible isolation of this population with only few exchanges of individuals with other populations from the same region. They can also indicate a previous population bottleneck or a founder effect following a recent colonization [7]. Indeed, the U.K. population of *A. imperator* was sampled near the city of York, at the Northern margin of its distribution range. In the current context of climate change [86], many species are shifting their distributions to higher altitudes or toward the poles [87]. A northward shift of range margins was already reported for the distribution of many odonates in England, including *A. imperator* that moved 85 km to the North between 1960–1970 and 1985–1995 periods [88]. Therefore, the observed low genetic diversity might be a consequence of a recent colonization of that sampling site. The two French populations from Bois-Guillaume and Heudreville also presented a significant degree of inbreeding. This was quite unexpected because these populations were among the largest that were sampled in the Normandy region. The pond in Bois-Guillaume is located in a suburban landscape suggesting a possible negative effect of the surrounding urbanization on exchanges between this population and the other ponds. Possible negative effects of human activities movements were already reported for other insect species [89,90]. The population from Heudreville was sampled in a large well-vegetated pond and with only few surrounding waterbodies [91] which may have prevented exchanges with other populations.

In pond networks, exchanges of individuals are a determining factor for the persistence of local populations. These networks are often referred to as metapopulations [92] in which some ponds act as sources or other as sinks [93]. In this study, the genetic variation was much higher within populations than between them, but a significant variation was found among countries. Among French (Normandy) populations, the genetic diversity was very similar. One of them (i.e., Bresle population) showed a genetic diversity very close to the U.K population, and another (i.e., Beaussault population) was closely related to the populations of Central and Southern European countries, especially the Swiss one. Within the French (Normandy) populations, one population (i.e., Cerisy) was identified as the main donor for all other populations, except the Bresle one, suggesting higher exchanges of this population with the U.K. population than with other Normandy populations. The Cerisy population was identified as a source of genetic variability for the rest of Normandy, whereas three other populations were only receivers of gene flow. The populations studied are probably supported by a network of many ponds. Therefore, we can hypothesize that the density of ponds around the Cerisy forest or the large size of the pond in Heudreville allows maintenance of large populations. From these source populations, some individuals may leave to smaller sink populations in areas where pond density is lower. However, this hypothesis requires further population demographic studies to be explored [94,95]. Overall, the results indicate a high gene flow in *A. imperator* between all sampled populations from continental Europe. These populations may be connected by long distance movements [96], but also by high rates of short distance movements between ponds, leading to a stepping-stone dispersal [97].

Increasing genetic isolation with distance is a common relationship that often shapes the genetic structure of populations [2]. At the European scale, this pattern was already reported for odonates (e.g., *Ischnura elegans*) [45], but also other flying insects (*Operophtera brumata* Linnaeus, 1958) [27] or flying mammals (*Myotis daubentonii* Kuhl, 1817) [98]. In the present study, this pattern was not found among the populations of *A. imperator* at the European scale. For instance, even if the U.K. population presented a genetic differentiation from the French (Normandy) populations located ca. 500 km away and populations

situated further east, no differentiation was found between the French populations and the populations of Central and Southern European countries. However, this result suggests that the English Channel could act as a physical barrier to the gene flow of *A. imperator* [99]. No genetic difference due to the English Channel or the Baltic Sea was found in smaller odonate species such as *I. elegans* [45]. This difference in results may be due to the fact that the populations of *I. elegans* can reach very high densities at some sites, which increases the observed intra-population variation and limits genetic drifts. For instance, in a survey of 20 ponds in Normandy, the larval density of *I. elegans*, was about 6 times higher than that of *A. imperator* [100]. The small populations of *A. imperator* may, therefore, be more prone to genetic differentiation in case of reduced gene flows [7].

Gene flows occurred from the U.K. population to one French population (i.e., Bresle) and from several French (Normandy) populations to all populations from Central and Southern European countries. These movements follow the same direction as the westerly winds brought by the gulf stream across the English Channel and prevailing in a major part of France. Whether by supporting active migrations of large species e.g., [101,102] or blowing small species through long distances [96], the wind probably plays a major role in the dispersal of odonates [38]. *Anax imperator* is a large and mobile dragonfly that was never reported as a migratory species, i.e., as a species flying on long-distances between emergence places and new habitats where reproduction take place [38]. However, we can hypothesize that some individuals might occasionally be able to undertake long-distance flights helped by wind currents, similarly to the regular migration movements described for the sister species *Anax junius* in the U.S.A [51]. A few rare long-distance movements supported by prevailing winds could cause some U.K. ponds to become sources of migrants for some French (Normand) ones, and other Normand ponds to become migration sources for further populations in Central and Southern European countries.

Genetic studies on odonates mostly use fresh material, especially legs of adults [43,44] or sometimes heads of adults [45,102]. However, this collection method is relatively invasive. It was recommended to avoid it for species with high conservation value [103] and negative effects on survival were observed on small odonate species on which several legs were lost [104]. Alternative methods based on DNA extraction from exuviae are, therefore, increasingly used [82,84]. This non-invasive method has the advantage of ensuring that individuals have grown in the studied site, while the origin cannot always be assessed for adults [105]. For larger species, collecting exuviae is also easier than catching flying adults that fly very fast over ponds during the reproductive period. Nevertheless, the persistence of DNA in these exoskeletons is poorly known. Especially, prolonged exposure to sunlight or enzymatic action on hydrated exuviae after rain may lead to a significant reduction of DNA yields [106]. In this study, we were able to compare the DNA yields of fresh exuviae reared at the laboratory with that of other 'old' exuviae sampled in situ. Although the total amount of DNA was much lower in 'old' exuviae, most samples (i.e., 31.6% of 'old' exuviae excluded versus 11.1% from 'fresh' exuviae excluded) could be used for this microsatellite study. We therefore recommend this method for further studies, at least on large species that are likely to contain more genetic material.

Legs of nymphs provide also a reliable source of DNA, but are still seldom used in population genetic studies on dragonflies but see [12,107]. Contrary to adults or exuviae, nymphs can be sampled during all weather conditions and all seasons of the year, a feature that can simplify the schedule of field sessions. Moreover, in many species, high nymphal densities ease the collection of a large number of samples, whereas flying adults may be hard to catch, especially those of Aeshnidae. Although identification of some species may be difficult in the field, we suggest that nymphal DNA sampling should be considered more in further studies, especially those on large dragonfly species.

Overall, this study provides insights into gene flow of *A. imperator* populations at both regional and European scales. Our results highlight the role of the English Channel as a potential barrier to dispersal, especially for movements from France (Normandy) to the U.K. They also suggest a probable role of the wind for long-distance movements of

odonates (e.g., Gulf Stream). Only a small fraction of ponds harboring *A. imperator* in Europe were sampled and more investigations would be useful to confirm the relationship between individuals and major wind currents. However, a high gene flow was found between continental populations, which may indicate that the distance between ponds at the European scale do not prevent dispersal movements of this large dragonfly. Dispersal probably occurs on a large spatial scale via successional movements from pond to pond at local scales. Nevertheless, the current pattern of genetic diversity may also mirror historical exchanges between populations rather than a contemporary gene flow [108]. Since the number of European ponds underwent a dramatic decline during the last century [34], the gene flow described in the present study may no longer be relevant today. Further studies comparing genetic markers with different mutation rates would be needed to address this question and disentangle historical and contemporary connectivity between European ponds [109].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14020068/s1, Figure S1: Estimation of number of clusters (K) with STRUCTURE using (a) mean of estimated Ln probabilities of data (±SD) for each K-value and (b) Delta K for each K-value (Evanno's method), Figure S2: Discriminant Analysis of Principal Components results of Anax imperator individuals of sampled French populations, Figure S3: Results of spatial Geneland analysis on the 11 populations of Anax imperator. Each figure corresponds to a cluster identified by Geneland. Black dots indicate the position of the populations (see Figure 1). Black lines indicated the posterior probabilities of membership in the three clusters, with darker colours (red) indicating highest posterior probabilities of belonging to the cluster.

Author Contributions: Conceptualization, M.M. and A.H.; methodology, M.M. and A.H.; field work and preparation of the samples: M.M.; laboratory work, M.M. and A.H.; software, A.H.; validation, M.M. and A.H.; formal analysis, A.H.; investigation, M.M. and A.H.; resources, A.H.; data curation, A.H.; writing—original draft preparation, M.M. and A.H.; writing—review and editing: M.M. and A.H.; supervision, A.H.; project administration, A.H.; funding acquisition, A.H. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Seine-Normandy Water Agency via the research project CROSS. M. Minot was supported by a grant provided by the French Ministry of Higher Education and Research through the Normandy's Doctoral School of Integrative Biology, Health and Environment (EdN BISE 497, Rouen University).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study will be publicly available from the corresponding author upon publication.

Acknowledgments: M. Aubert and A. Husté (ECODIV, EA 1293 INRAe) were the co-supervisors. We want to thank the contributors that accepted to send us samples for this study. Thanks are especially due to Andrea Corso from Sicily (Italy), Sebastien Tschanz from Neuchâtel (Switzerland) and Martin Roberts from York (United Kingdom). We also thank L. Mundell for reviewing the English language.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thomas, C.D.; Kunin, W.E. The Spatial Structure of Populations. *J. Anim. Ecol.* **1999**, *68*, 647–657. [CrossRef]
2. Sexton, J.P.; Hangartner, S.B.; Hoffmann, A.A. Genetic Isolation by Environment or Distance: Which Pattern of Gene Flow Is Most Common? *Evolution* **2013**, *68*, 1–15. [CrossRef]
3. Norman, A.J.; Stronen, A.V.; Fuglstad, G.-A.; Ruiz-Gonzalez, A.; Kindberg, J.; Street, N.R.; Spong, G. Landscape Relatedness: Detecting Contemporary Fine-Scale Spatial Structure in Wild Populations. *Landsc. Ecoogyl.* **2017**, *32*, 181–194. [CrossRef]
4. Maxwell, S.L.; Fuller, R.A.; Brooks, T.M.; Watson, J.E. Biodiversity: The Ravages of Guns, Nets and Bulldozers. *Nat. News* **2016**, *536*, 143–145. [CrossRef] [PubMed]
5. Frankham, R.; Ballou, J.D.; Briscoe, D.A. *Introduction to Conservation Genetics*; Cambridge University Press: Cambridge, UK, 2010.
6. Dennis, R.L.H.; Dapporto, L.; Dover, J.W.; Shreeve, T.G. Corridors and Barriers in Biodiversity Conservation: A Novel Resource-Based Habitat Perspective for Butterflies. *Biodivers. Conserv.* **2013**, *22*, 2709–2734. [CrossRef]

7. Allendorf, F.W.; Luikart, G.H.; Aitken, S.N. *Conservation and the Genetics of Populations*, 2nd ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2012.
8. Bergamaschi, A.C.B.; Lama, M.A.D.; Bergamaschi, A.C.B.; Lama, M.A.D. Gene Variation and Genetic Differentiation among Populations of the Solitary Mud Dauber Wasp *Trypoxylon* (*Trypargilum*) *albitarse* Fabricius 1804 (Hymenoptera, Crabronidae). *Genet. Mol. Biol.* **2015**, *38*, 519–526. [CrossRef]
9. De Meester, L.; Gómez, A.; Okamura, B.; Schwenk, K. The Monopolization Hypothesis and the Dispersal–Gene Flow Paradox in Aquatic Organisms. *Acta Oecologica* **2002**, *23*, 121–135. [CrossRef]
10. Habel, J.C.; Junker, M.; Schmitt, T. High Dispersal Ability and Low Genetic Differentiation in the Widespread Butterfly Species *Melanargia galathea*. *J. Insect Conserv.* **2010**, *14*, 467–478. [CrossRef]
11. Heinken, T.; Weber, E. Consequences of Habitat Fragmentation for Plant Species: Do We Know Enough? Perspectives in Plant Ecology. *Evol. Syst.* **2013**, *15*, 205–216. [CrossRef]
12. Kohli, M.K.; Sahlén, G.; Kuhn, W.R.; Ware, J.L. Extremely Low Genetic Diversity in a Circumpolar Dragonfly Species, *Somatochlora sahlbergi* (Insecta: Odonata: Anisoptera). *Sci. Rep.* **2018**, *8*, 15114. [CrossRef]
13. Baguette, M.; Blanchet, S.; Legrand, D.; Stevens, V.M.; Turlure, C. Individual Dispersal, Landscape Connectivity and Ecological Networks. *Biol. Rev.* **2013**, *88*, 310–326. [CrossRef]
14. Jordano, P. What Is Long-Distance Dispersal? And a Taxonomy of Dispersal Events. *J. Ecol.* **2017**, *105*, 75–84. [CrossRef]
15. Le Corre, V.; Kremer, A. Cumulative Effects of Founding Events during Colonisation on Genetic Diversity and Differentiation in an Island and Stepping-Stone Model. *J. Evol. Biol.* **1998**, *11*, 495–512. [CrossRef]
16. Coughlan, N.E.; Kelly, T.C.; Jansen, M.A.K. "Step by Step": High Frequency Short-Distance Epizoochorous Dispersal of Aquatic Macrophytes. *Biol. Invasions* **2017**, *19*, 625–634. [CrossRef]
17. Nathan, R. Long-Distance Dispersal of Plants. *Science* **2006**, *313*, 786–788. [CrossRef]
18. Da Fonte, L.F.M.; Mayer, M.; Lötters, S. Long-Distance Dispersal in Amphibians. *Front. Biogeogr.* **2019**, *11*, 1–14. [CrossRef]
19. Newton, I. *The Migration Ecology of Birds*; Elsevier/Acad. Press: Amsterdam, The Netherlands, 2008.
20. Brink, K.; Gough, P.; Royte, J.; Schollema, P.P.; Wanningen, H. *From Sea to Source 2.0. Protection and Restoration of Fish Migration in Rivers Worldwide*; World Fish Migration Foundation: Groningen, The Netherlands, 2018.
21. Bowen, B.W.; Karl, S.A. Population Genetics and Phylogeography of Sea Turtles. *Mol. Ecol.* **2007**, *16*, 4886–4907. [CrossRef]
22. Haest, B.; Hüppop, O.; van de Pol, M.; Bairlein, F. Autumn Bird Migration Phenology: A Potpourri of Wind, Precipitation and Temperature Effects. *Glob. Chang. Biol.* **2019**, *25*, 4064–4080. [CrossRef]
23. Green, A.J.; Figuerola, J. Recent Advances in the Study of Long-Distance Dispersal of Aquatic Invertebrates via Birds. *Divers. Distrib.* **2005**, *11*, 149–156. [CrossRef]
24. Stockwell, C.A.; Hendry, A.P.; Kinnison, M.T. Contemporary Evolution Meets Conservation Biology. *Trends Ecol. Evol.* **2003**, *18*, 94–101. [CrossRef]
25. Kelly, E.; Phillips, B.L. Targeted gene flow for conservation. *Conserv. Biol.* **2016**, *30*, 259–267. [CrossRef] [PubMed]
26. Ross, K.G. How to Measure Dispersal: The Genetic Approach. The Example of Fire Ants. In *Dispersal*; Oxford University Press: Oxford, NY, USA, 2001; pp. 29–42.
27. Leggett, H.C.; Jones, E.O.; Burke, T.; Hails, R.S.; Sait, S.M.; Boots, M. Population Genetic Structure of the Winter Moth, *Operophtera brumata* Linnaeus, in the Orkney Isles Suggests Long-Distance Dispersal. *Ecol. Entomol.* **2011**, *36*, 318–325. [CrossRef]
28. Biggs, J.; von Fumetti, S.; Kelly-Quinn, M. The Importance of Small Waterbodies for Biodiversity and Ecosystem Services: Implications for Policy Makers. *Hydrobiologia* **2017**, *793*, 3–39. [CrossRef]
29. Hill, M.J.; Biggs, J.; Thornhill, I.; Briers, R.A.; Gledhill, D.G.; White, J.C.; Wood, P.J.; Hassall, C. Urban Ponds as an Aquatic Biodiversity Resource in Modified Landscapes. *Glob. Chang. Biol.* **2017**, *23*, 986–999. [CrossRef]
30. Kimura, M.; Weiss, G.H. The Stepping Stone Model of Population Structure and the Decrease of Genetic Correlation with Distance. *Genetics* **1964**, *49*, 561–576. [CrossRef]
31. Watts, A.G.; Schlichting, P.; Billerman, S.; Jesmer, B.; Micheletti, S.; Fortin, M.-J.; Funk, C.; Hapeman, P.; Muths, E.L.; Murphy, M.A. How Spatio-Temporal Habitat Connectivity Affects Amphibian Genetic Structure. *Front. Genet.* **2015**, *6*, 1–13. [CrossRef] [PubMed]
32. Youngquist, M.B.; Inoue, K.; Berg, D.J.; Boone, M.D. Effects of Land Use on Population Presence and Genetic Structure of an Amphibian in an Agricultural Landscape. *Landsc. Ecol.* **2017**, *32*, 147–162. [CrossRef]
33. Thornhill, I.; Batty, L.; Hewitt, M.; Friberg, N.R.; Ledger, M.E. The Application of Graph Theory and Percolation Analysis for Assessing Change in the Spatial Configuration of Pond Networks. *Urban Ecosyst.* **2018**, *21*, 213–225. [CrossRef]
34. Oertli, B.; Biggs, J.; Céréghino, R.; Grillas, P.; Joly, P.; Lachavanne, J.-B. Conservation and Monitoring of Pond Biodiversity: Introduction. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2005**, *15*, 535–540. [CrossRef]
35. Anderson, T.L.; Ousterhout, B.H.; Peterman, W.E.; Drake, D.L.; Semlitsch, R.D. Life History Differences Influence the Impacts of Drought on Two Pond-Breeding Salamanders. *Ecol. Appl.* **2015**, *25*, 1896–1910. [CrossRef]
36. Suhling, F.; Sahlén, G.; Gorb, S.; Kalkman, V.J.; Dijkstra, K.-D.B.; van Tol, J. Order Odonata. In *Thorp and Covich's Freshwater Invertebrates*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 893–932. [CrossRef]
37. Bilton, D.T.; Freeland, J.R.; Okamura, B. Dispersal in Freshwater Invertebrates. *Annu. Rev. Ecol. Syst.* **2001**, *32*, 159–181. [CrossRef]
38. Corbet, P.S. *Dragonflies: Behaviour and Ecology of Odonata*; Harley Books: Colchester, UK, 1999.

39. Suhling, F.; Martens, A.; Suhling, I. Long-Distance Dispersal in Odonata: Examples from Arid Namibia. *Austral Ecol.* **2017**, *42*, 544–552. [CrossRef]
40. Conrad, K.F.; Willson, K.H.; Harvey, I.F.; Thomas, C.J.; Sherratt, T.N. Dispersal Characteristics of Seven Odonate Species in an Agricultural Landscape. *Ecography* **1999**, *22*, 524–531. [CrossRef]
41. Rouquette, J.R.; Thompson, D.J. Patterns of Movement and Dispersal in an Endangered Damselfly and the Consequences for Its Management. *J. Appl. Ecol.* **2007**, *44*, 692–701. [CrossRef]
42. Le Gall, M.; Chaput-Bardy, A.; Husté, A. Context-Dependent Local Movements of the Blue-Tailed Damselfly, *Ischnura elegans*: Effects of Pond Characteristics and the Landscape Matrix. *J. Insect Conserv.* **2017**, *21*, 243–256. [CrossRef]
43. Troast, D.; Suhling, F.; Jinguji, H.; Sahlén, G.; Ware, J. A Global Population Genetic Study of *Pantala flavescens*. *PLoS ONE* **2016**, *11*, e0148949. [CrossRef] [PubMed]
44. Lorenzo-Carballa, M.O.; Ferreira, S.; Sims, A.M.; Thompson, D.J.; Watts, P.C.; Cher, Y.; Damoy, V.; Evrard, A.; Gelez, W.; Vanappelghem, C. Impact of Landscape on Spatial Genetic Structure and Diversity of *Coenagrion mercuriale* (Zygoptera:Coenagrionidae) in Northern France. *Freshw. Sci.* **2015**, *34*, 1065–1078. [CrossRef]
45. Wellenreuther, M.; Sánchez-Guillén, R.A.; Cordero-Rivera, A.; Svensson, E.I.; Hansson, B. Environmental and Climatic Determinants of Molecular Diversity and Genetic Population Structure in a Coenagrionid Damselfly. *PLoS ONE* **2011**, *6*, e20440. [CrossRef]
46. Lorenzo-Carballa, M.O.; Hadrys, H.; Cordero-Rivera, A.; Andrés, J.A. Population Genetic Structure of Sexual and Parthenogenetic Damselflies Inferred from Mitochondrial and Nuclear Markers. *Heredity* **2012**, *108*, 386–395. [CrossRef] [PubMed]
47. Purse, B.V.; Hopkins, G.W.; Day, K.J.; Thompson, D.J. Dispersal Characteristics and Management of a Rare Damselfly. *J. Appl. Ecol.* **2003**, *40*, 716–728. [CrossRef]
48. Watts, P.C.; Saccheri, I.J.; Kemp, S.J.; Thompson, D.J. Population Structure and the Impact of Regional and Local Habitat Isolation upon Levels of Genetic Diversity of the Endangered Damselfly *Coenagrion mercuriale* (Odonata: Zygoptera). *Freshw. Biol.* **2006**, *51*, 193–205. [CrossRef]
49. Boudot, J.P.; Kalkman, V.J. *Atlas of the European Dragonflies and Damselflies*; KNNV Publishing: Zeist, The Netherlands, 2015.
50. Flenner, I.; Sahlén, G. Dragonfly community re-organisation in boreal forest lakes: Rapid species turnover driven by climate change? *Insect Conserv. Divers.* **2008**, *1*, 169–179. [CrossRef]
51. Knight, S.M.; Pitman, G.M.; Flockhart, D.T.T.; Norris, D.R. Radio-Tracking Reveals How Wind and Temperature Influence the Pace of Daytime Insect Migration. *Biol. Lett.* **2019**, *15*, 20190327. [CrossRef]
52. Minot, M.; Le Gall, M.; Husté, A. Biometry of the Large Dragonfly *Anax imperator* (Odonata: Aeshnidae): A Study of Traits from Larval Development to Adults. *Eur. J. Entomol.* **2019**, *116*, 269–280. [CrossRef]
53. Corbet, P.S. The Life-History of the Emperor Dragonfly *Anax imperator* Leach (Odonata: Aeshnidae). *J. Anim. Ecol.* **1957**, *26*, 1–69. [CrossRef]
54. Minot, M.; Besnard, A.; Husté, A. Habitat Use and Movements of a Large Dragonfly (Odonata: *Anax Imperator*) in a Pond Network. *Freshw. Biol.* **2021**, *66*, 241–255. [CrossRef]
55. Lubertazzi, M.A.A.; Ginsberg, H.S. Persistence of Dragonfly Exuviae on Vegetation and Rock Substrates. *Northeast. Nat.* **2009**, *16*, 141–147. [CrossRef]
56. Roland, H.-J. Haltbarkeit von Anax-Exuvien Am Ort Der Emergenz. *Libellula* **2010**, *3*, 231–240.
57. Keller, D.; Brodbeck, S.; Holderegger, R. Characterization of Microsatellite Loci in *Leucorrhinia caudalis*, a Rare Dragonfly Endangered throughout Europe. *Conserv. Genet. Resour.* **2009**, *1*, 179. [CrossRef]
58. Hadrys, H.; Timm, J.; Streit, B.; Giere, S. A Panel of Microsatellite Markers to Study Sperm Precedence Patterns in the Emperor Dragonfly *Anax imperator* (Odonata: Anisoptera). *Mol. Ecol. Notes* **2007**, *7*, 296–298. [CrossRef]
59. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
60. Adamack, A.T.; Gruber, B. Landgenreport: A new R function to simplify landscape genetic analysis using resistance surface layers. *Mol. Ecol. Resour.* **2015**, *15*, 1172–1178.
61. Paradis, E. Pegas: An R Package for Population Genetics with an Integrated–Modular Approach. *Bioinformatics* **2010**, *26*, 419–420. [CrossRef] [PubMed]
62. Raymond, M.; Rousset, F. An Exact Test for Population Differentiation. *Evolution* **1995**, *49*, 1280–1283. [CrossRef]
63. Rousset, F. Genepop'007: A Complete Re-Implementation of the Genepop Software for Windows and Linux. *Mol. Ecol. Resour.* **2008**, *8*, 103–106. [CrossRef]
64. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B (Methodol.)* **1995**, *57*, 289–300. [CrossRef]
65. Benjamini, Y.; Yekutieli, D. The Control of the False Discovery Rate in Multiple Testing under Dependency. *Ann. Stat.* **2001**, *29*, 1165–1188. [CrossRef]
66. Keenan, K.; McGinnity, P.; Cross, T.F.; Crozier, W.W.; Prodöhl, P.A. DiveRsity: An R Package for the Estimation of Population Genetics Parameters and Their Associated Errors. *Methods Ecol. Evol.* **2013**, *4*, 782–788. [CrossRef]
67. Kalinowski, S.T. Counting Alleles with Rarefaction: Private Alleles and Hierarchical Sampling Designs. *Conserv. Genet.* **2004**, *5*, 539–543. [CrossRef]

68. Gaggiotti, O.E.; Lange, O.; Rassmann, K.; Gliddon, C. A Comparison of Two Indirect Methods for Estimating Average Levels of Gene Flow Using Microsatellite Data. *Mol. Ecol.* **1999**, *8*, 1513–1520. [CrossRef] [PubMed]
69. Whitlock, M.C.; Mccauley, D.E. Indirect Measures of Gene Flow and Migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity* **1999**, *82*, 117–125. [CrossRef]
70. Weir, B.S.; Cockerham, C.C. Estimating F-Statistics for the Analysis of Population Structure. *Evolution* **1984**, *38*, 1358–1370.
71. Excoffier, L.; Smouse, P.E.; Quattro, J.M. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics* **1992**, *131*, 479–491. [CrossRef] [PubMed]
72. Rousset, F. Genetic Differentiation and Estimation of Gene Flow from F-Statistics under Isolation by Distance. *Genetics* **1997**, *145*, 1219–1228. [CrossRef]
73. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
74. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
75. Jakobsson, M.; Rosenberg, N.A. CLUMPP: A Cluster Matching and Permutation Program for Dealing with Label Switching and Multimodality in Analysis of Population Structure. *Bioinformatics* **2007**, *23*, 1801–1806. [CrossRef]
76. Archer, F.I.; Adams, P.E.; Schneiders, B.B. Stratag: An r Package for Manipulating, Summarizing and Analysing Population Genetic Data. *Mol. Ecol. Resour.* **2017**, *17*, 5–11. [CrossRef]
77. Jombart, T. Adegenet: A R Package for the Multivariate Analysis of Genetic Markers. *Bioinformatics* **2008**, *24*, 1403–1405. [CrossRef]
78. Guillot, G.; Mortier, F.; Estoup, A. Geneland: A Computer Package for Landscape Genetics. *Mol. Ecol. Notes* **2005**, *5*, 712–715. [CrossRef]
79. Blair, C.; Weigel, D.E.; Balazik, M.; Keeley, A.T.; Walker, F.M.; Landguth, E.; Cushman, S.A.M.; Murphy, M.; Waits, L.; Balkenhol, N. A Simulation-Based Evaluation of Methods for Inferring Linear Barriers to Gene Flow. *Mol. Ecol. Resour.* **2012**, *12*, 822–833. [CrossRef]
80. Wilson, G.A.; Rannala, B. Bayesian Inference of Recent Migration Rates Using Multilocus Genotypes. *Genetics* **2003**, *163*, 1177–1191. [CrossRef]
81. Gamerman, D. *Markov Chain Monte Carlo: Stochastic Simulation for Bayesian Inference*; Chapman & Hall: New York, NY, USA, 1997.
82. Keller, D.; Brodbeck, S.; Flöss, I.; Vonwil, G.; Holderegger, R. Ecological and Genetic Measurements of Dispersal in a Threatened Dragonfly. *Biol. Conserv.* **2010**, *143*, 2658–2663. [CrossRef]
83. Dolný, A.; Šigutová, H.; Ožana, S.; Choleva, L. How Difficult Is It to Reintroduce a Dragonfly? Fifteen Years Monitoring *Leucorrhinia dubia* at the Receiving Site. *Biol. Conserv.* **2018**, *218*, 110–117. [CrossRef]
84. Swaegers, J.; Janssens, S.B.; Ferreira, S.; Watts, P.C.; Mergeay, J.; McPeek, M.A.; Stoks, R. Ecological and Evolutionary Drivers of Range Size in *Coenagrion* Damselflies. *J. Evol. Biol.* **2014**, *27*, 2386–2395. [CrossRef]
85. Levett, S.; Walls, S. Tracking the Elusive Life of the Emperor Dragonfly *Anax imperator* Leach. *J. Br. Dragonfly Soc.* **2011**, *27*, 59–68.
86. IPCC Climate Change 2013: The Physical Science Basis. *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013.
87. Hickling, R.; Roy, D.B.; Hill, J.K.; Fox, R.; Thomas, C.D. The Distributions of a Wide Range of Taxonomic Groups Are Expanding Polewards. *Glob. Chang. Biol.* **2006**, *12*, 450–455. [CrossRef]
88. Hickling, R.; Roy, D.B.; Hill, J.K.; Thomas, C.D. A Northward Shift of Range Margins in British Odonata. *Glob. Chang. Biol.* **2005**, *11*, 502–506. [CrossRef]
89. Jha, S. Contemporary Human-Altered Landscapes and Oceanic Barriers Reduce Bumble Bee Gene Flow. *Mol. Ecol.* **2015**, *24*, 993–1006. [CrossRef]
90. Vanden Broeck, A.; Maes, D.; Kelager, A.; Wynhoff, I.; WallisDeVries, M.F.; Nash, D.R.; Oostermeijer, J.G.B.; Van Dyck, H.; Mergeay, J. Gene Flow and Effective Population Sizes of the Butterfly *Maculinea alcon* in a Highly Fragmented, Anthropogenic Landscape. *Biol. Conserv.* **2017**, *209*, 89–97. [CrossRef]
91. PRAM Normandie. Available online: http://recensementmare.pramnormandie.com/API/ (accessed on 11 January 2022).
92. Gilpin, E.M.; Hanski, I. Metapopulation Dynamics: Empirical and Theoretical Investigations. *Biol. J. Linn. Soc.* **1991**, *42*, 3–16.
93. Pulliam, H.R. Sources, Sinks, and Population Regulation. *Am. Nat.* **1988**, *132*, 652–661. [CrossRef]
94. Peery, M.Z.; Beissinger, S.R.; House, R.F.; Bérubé, M.; Hall, L.A.; Sellas, A.; Palsbøll, P.J. Characterizing Source–Sink Dynamics with Genetic Parentage Assignments. *Ecology* **2008**, *89*, 2746–2759. [CrossRef] [PubMed]
95. Thomaz, A.T.; He, Q. When Are Populations Not Connected like a Circuit? Identifying Biases in Gene Flow from Coalescent Times. *Mol. Ecol. Resour.* **2019**, *19*, 1381–1384. [CrossRef]
96. Suhling, F.; Martens, A.; Marais, E. How to Enter a Desert—Patterns of Odonata Colonisation of Arid Namibia. *Int. J. Odonatol.* **2009**, *12*, 287–308. [CrossRef]
97. Saura, S.; Bodin, Ö.; Fortin, M.-J. EDITOR'S CHOICE: Stepping Stones Are Crucial for Species' Long-Distance Dispersal and Range Expansion through Habitat Networks. *J. Appl. Ecol.* **2014**, *51*, 171–182. [CrossRef]
98. Atterby, H.; Aegerter, J.N.; Smith, G.C.; Conyers, C.M.; Allnutt, T.R.; Ruedi, M.; MacNicoll, A.D. Population Genetic Structure of the Daubenton's Bat (*Myotis daubentonii*) in Western Europe and the Associated Occurrence of Rabies. *Eur. J. Wildl. Resour.* **2010**, *56*, 67–81. [CrossRef]

99. Razgour, O.; Rebelo, H.; Puechmaille, S.J.; Juste, J.; Ibáñez, C.; Kiefer, A.; Burke, T.; Dawson, D.A.; Jones, G. Scale-Dependent Effects of Landscape Variables on Gene Flow and Population Structure in Bats. *Divers. Distrib.* **2014**, *20*, 1173–1185. [CrossRef]
100. Minot, M.; Aubert, M.; Husté, A. Pond Creation and Restoration: Patterns of Odonate Colonization and Community Dynamics. *Biodivers. Conserv.* **2021**, *30*, 4379–4399. [CrossRef]
101. May, M.L. A Critical Overview of Progress in Studies of Migration of Dragonflies (Odonata: Anisoptera), with Emphasis on North America. *J. Insect Conserv.* **2013**, *17*, 1–15. [CrossRef]
102. Chaput-Bardy, A.; Lemaire, C.; Picard, D.; Secondi, J. In-Stream and Overland Dispersal across a River Network Influences Gene Flow in a Freshwater Insect, *Calopteryx splendens*. *Mol. Ecol.* **2008**, *17*, 3496–3505. [CrossRef] [PubMed]
103. Monroe, E.M.; Lynch, C.; Soluk, D.A.; Britten, H.B. Nonlethal Tissue Sampling Techniques and Microsatellite Markers Used for First Report of Genetic Diversity in Two Populations of the Endangered *Somatochlora hineana* (Odonata: Corduliidae). *Ann. Entomol. Soc. Am.* **2010**, *103*, 1012–1017. [CrossRef]
104. Cordero-Rivera, A.; Perez, F.E.; Andres, J.A. The Effect of Handling Damage, Mobility, Body Size, and Fluctuating Asymmetry on Lifetime Mating Success of *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica* **2002**, *31*, 117–128.
105. Raebel, E.M.; Merckx, T.; Riordan, P.; Macdonald, D.W.; Thompson, D.J. The Dragonfly Delusion: Why It Is Essential to Sample Exuviae to Avoid Biased Surveys. *J. Insect Conserv.* **2010**, *14*, 523–533. [CrossRef]
106. Watts, P.C.; Thompson, D.J.; Daguet, C.; Kemp, S.J. Exuviae as a Reliable Source of DNA for Population-Genetic Analysis of Odonates. *Odonatologica* **2005**, *34*, 183–187.
107. Monroe, E.M.; Britten, H.B. Conservation in Hine's Sight: The Conservation Genetics of the Federally Endangered Hine's Emerald Dragonfly, *Somatochlora hineana*. *J. Insect Conserv.* **2014**, *18*, 353–363. [CrossRef]
108. Incagnone, G.; Marrone, F.; Barone, R.; Robba, L.; Naselli-Flores, L. How Do Freshwater Organisms Cross the "Dry Ocean"? A Review on Passive Dispersal and Colonization Processes with a Special Focus on Temporary Ponds. *Hydrobiologia* **2015**, *750*, 103–123. [CrossRef]
109. Epps, C.W.; Keyghobadi, N. Landscape Genetics in a Changing World: Disentangling Historical and Contemporary Influences and Inferring Change. *Mol. Ecol.* **2015**, *24*, 6021–6040. [CrossRef] [PubMed]