Connectivity within isolation: dispersal, population genetics, and conservation of the rarest European damselfly

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Abstract. 1. Coenagrion hylas (Trybom, 1899) has a very limited distribution in Europe, lives in very small, isolated populations, has rather specialised habitat demands, and is regarded as the rarest damselfly of Europe.

2. Using a combination of capture-mark-recapture and population genetics, we aimed to evaluate the state of the populations in the Tyrolean Lech valley and to test whether exuviae from this species are usable as a DNA source. DNA was extracted from mid-leg tibiae and exuviae and genotyped with species-specific microsatellite markers. The results from the capture-recapture and the genetic methods were congruent.

3. Coenagrion hylas has an unexpectedly high tendency to disperse within the valley, covering distances of up to 30 km, and lives longer than other damselflies, with an average longevity of 12 days and a maximum lifespan of at least 40 days. Low inbreeding coefficients and low ranges of genetic differentiation across sites provide evidence of panmixia, with no clear signs of inbreeding. The current population size is estimated at 1150 males based on the recapture data.

4. We further demonstrated that exuviae deliver a sufficient amount of DNA, which will be important for future monitoring. Although C. hylas currently shows appropriate viability at most Lech valley sites, our study indicates that management measurements, such as creating stepping stone habitats, are crucial to maintain the current population status. Given the high dispersal capability of the species, such management measurements seem promising.

Key words. Capture-mark-recapture, Coenagrion hylas, exuviae, gene flow, longevity, microsatellites, Odonata.

Introduction

Many formerly widespread species of dragonflies have shrunk in numbers of populations and individuals (Keller et al., 2010) due to the endangerment of freshwater ecosystems in Europe during the last decades (Dudgeon et al., 2006). The problem is reflected in the high portions of odonates classified as ‘threatened’ (VU), ‘endangered’ (EN), and even as ‘critically endangered’ (CR) in the Red Lists of Europe [15% VU–CR, 11% near threatened (NT); Kalkman et al., 2010]. Threat assessments are even more alarming for highly industrialised Central European countries like Austria (57% VU–CR, 10% NT; Raab, 2006), Germany (38% VU–CR, 6% VU–CR; Ott et al., 2015), and Switzerland (36% VU–CR, 17% NT; Gonseth & Monnerat, 2002). There are signs of a recent recovery and increase, particularly for some lotic species as well as for species of southern provenience as an effect of climate warming and habitat improvements (Ott et al., 2015; Termaat et al., 2015).

However, two ecological groups among odonates are still of special conservation concern both on international and national scales. These ecological groups are represented in disproportionately high numbers and in higher threat categories in the Red Lists...
cited: habitat specialists bound to moorland pools and peat bog habitats (Termaat et al., 2015) and lotic or rheophilic species bound to landscapes with a network of undisturbed or near-natural running waters (Kalkman et al., 2010). Moreover, especially for populations considered to be (postglacial) relics, habitat loss caused by humans is regarded to be particularly severe (Habel et al., 2010).

In this study, we deal with the zygopteran damselfly Coenagrion hylas (Trybom, 1899), an East Siberian species regarded to be a postglacial relic in Europe (Bernard & Daraž, 2010). As a representative of a cold-stenothermal fauna, this species is confined to small bog lakes surrounded by fens and peat-bogs and to spring-water ponds, both of which exhibit inflow of cold, slow running water (Landmann et al., 2005; Müller, 2015).

Coenagrion hylas has a very limited distribution in Europe, and its populations are extremely small and isolated. Apart from a few sites in the Ural and Pinega region of Russia, vital populations in Europe currently are exclusively known from an area of occurrence limited to about 42 km² at the Northern Carpathian Alps of Tyrol, Austria. Even there C. hylas has been recorded at only two dozen sites, which were only partly and/or irregularly occupied by the species in the last 50 years (Landmann et al., 2005; Landmann, 2013; Wildermuth & Martens, 2019). The species therefore is widely considered to be Europe’s rarest damselfly and is stated as vulnerable in the IUCN Red List (Kalkman et al., 2010). Furthermore, it is listed in Annex II of the EU Habitats directive (EU-FFH). The species thus clearly deserves special attention and protection on an international scale.

The few known sites of occurrence of this species in the Alps are separated from each other by high mountain chains and other barriers like it is the case in our investigation area, the EU-Natura 2000 area ‘Lechtal’, which can be regarded as the actual European stronghold of the species.

Within a fragmented landscape, such barriers are widely regarded as a main factor promoting biodiversity loss (Lindenmayr & Fischer, 2006), in particular because they can disturb connectivity among populations. An important effect of dispersal is the avoidance or reduction of inbreeding (Waser et al., 1986; Perrin & Mazalov, 1999). Both habitat fragmentation and lack of exchange are the main problems in maintaining a (genetically) stable population that is likely to persist. This is especially true when considering the conservation of small and endangered insect species like damselflies. Damselflies mostly have restricted dispersal abilities of only a few hundred metres up to less than 7 km and very short life expectancy of up to 10 days (Purse et al., 2003; Beirinckx et al., 2006; Rouquette & Thompson, 2007; Allen & Thompson, 2010; Alp et al., 2012; Hassall & Thompson, 2012; Keller & Holderegger, 2013).

While many management strategies – for invertebrates in general and odonates in particular – traditionally focus on habitat conservation and restoration (Wildermuth & Küry, 2009), less is known about the actual connectivity of populations of damselflies. Although monitoring and conservation programmes for C. hylas have been put forward in the course of a first LIFE-Project in the Natura 2000 area Lechtal (Müller, 2001), nothing has been known about the genetic diversity and connectivity of the mostly small and isolated subpopulations of this species in the Alps until today. However, such information is crucial for further conservation and management strategies for C. hylas. In particular, knowledge about the dispersal ability of the species is imperative to assess the suitability of expensive conservation measures like the building and restoring of stepping stones and habitats for reproduction at appropriate distances to ensure functional connectivity between subpopulations.

Dispersal and concomitant connectivity of meta-populations can be investigated either directly with methods like mark-recapture or indirectly with the help of genetic methods. Capture-mark-recapture studies are the classic approach, with some drawbacks concerning accurate detection of previously marked animals or underestimating dispersal distances, especially in odonates (Keller et al., 2010; Hassall & Thompson, 2012). In contrast, genetic methods can help not only to cover for those drawbacks but also give a more detailed look into species-specific dispersal patterns (Hughes et al., 2009).

For dragonfly species, sampling exuviae would be a minimum-invasive method to gain DNA for genetic methods, but the poor DNA yield has sparked controversial debates about the suitability of exuviae for DNA extraction (Ozana et al., 2020; Watts et al., 2005b). Hence, more invasive methods such as extracting tibiae of adult individuals are used preferably to gather sufficient amounts of DNA (Monroe et al., 2010).

Since the combination of basic and molecular methods has proven to provide deeper insight in the actual state of insects from various orders (Oi et al., 2013; Tait et al., 2018), we both conducted an extensive capture-mark-recapture study and used newly designed microsatellite markers (see Landmann et al., 2021) to investigate the state of several subpopulations as the basis for a sound conservation and management for C. hylas in the Natura 2000 area Lechtal.

This study aims to:

1. investigate dispersal abilities of C. hylas within the known subpopulations, spread over the Lech river valley, with both capture-mark-recapture and molecular methods;
2. explore the genetic diversity and potential signs of inbreeding among and within the subpopulations of C. hylas; and
3. verify if non-invasive sampling by using damselfly exuviae would result in equally adequate DNA yields as using midleg tibiae.

Material and methods

Study species

Coenagrion hylas is a comparatively stout damselfly in both sexes. With a body size of up to 4 cm and a dry mass of about 7 mg (Swaegers et al., 2014; Wildermuth & Martens, 2019), it is the largest of all European coenagrionid species and can readily be distinguished by its distinct colouration even from some distance with the aid of binoculars. As the species is also relatively strong winged (see Swaegers et al., 2014), wing marking and controls of individuals in the field are practical. Coenagrion hylas therefore is a good species for catch-recapture/catch-resight studies.

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Study area, study sites, and sample collection

The investigation area is the EU-Natura 2000 area Lechtal, Tyrol, Austria (Fig. 1). Since the year 2000, *C. hylas* has been known to occur at about a dozen small water bodies, which are spaced out over a stretch of approximately 30 km along the Lech river, and adjoining terraces at altitudes between 890 and 1200 m above sea level. These water bodies are separated and isolated from each other by uninhabited alluvial habitats, forests, and agricultural land as well as by altitudinal and other barriers.

The habitat demands of *C. hylas* are rather specialised (Landmann et al., 2005; Müller, 2015). In the study area, the species is confined to small spring-water ponds and swamps, some shallow floodplain waters, and two small boggy lakes with clear oligotrophic water. Most sites include zones of cold shallow, slow running, and seepage water. Based on measurements using high-resolution aerial photos, the size of the water bodies used by *C. hylas* for reproduction in the study area ranges from 0.05 to 0.30 ha.

In 2019, all known sites were visited, and all sites where *C. hylas* was found were monitored regularly. The species was recorded at 10 sites (Fig. 1), which were then visited in a 3-to-5-day-rhythm under good weather conditions. This resulted in a total of 29 field days from mid-May to the end of July. Both exuviae and mid-leg tibiae of adult individuals were collected for genetic analyses. One tibia per individual was cut off with cuticle scissors, immediately placed in 96% ethanol, and stored at \(-20 ^\circ C\) until further processing. Exuviae were placed in dry collection tubes and immediately stored in liquid nitrogen (dry shipper) until transfer into a \(-80 ^\circ C\) freezer in the laboratory.

Capture-mark-recapture study and analysis

We carried out a mark-recapture study at all 10 sites. Marking started at the first encounter with an adult damselfly, on June 11th, and continued until the last day of sampling, on July 23rd. Recapturing was done on each sampling day. Mature adult damselflies were caught with a hand net and marked on the hindwing with an individual consecutive number using a Multi-mark 1523 permanent marker (Faber-Castell, Germany). Overall, 17 capture-mark-recapture occasions were performed during these 29 field days. GPS data of the first encounter and all recatches were recorded for each individual. In total, tibiae of 304 individuals (279 males, 25 females) and 112 exuviae (54 m, 58 f) were collected, and 507 individuals (469 m, 38 f) were marked.

A raw calculation for the average longevity of males was performed manually. Mark-recapture data were further processed in MARK v9.0 (White & Burnham, 1999) to calculate population sizes for the whole Lech valley. We used the Jolly-Seber method in the POPAN parameterisation for open population estimates (Lebreton et al., 1992). The POPAN formulation estimates four primary parameters: (i) \(\phi_i\), the probability of survival between two occasions; (ii) \(p_i\), the catchability on day \(i\); (iii) \(p_{\text{ent}}\), the
Table 1. Sample size: Number of individuals used for genetic analyses and of marked and recaptured males and females per study site (see Fig. 1). Prior to genetic analyses, tibiae samples with a certain percentage of failures (more than 20% for sites 1, 3, 5, 9; more than 30% for Site 2 and more than 40% for Site 4) were filtered out. After filtering, we chose 219 individuals to be the remaining source for further genetic analyses. Row 4a refers to tibiae, row 4b to exuviae of Site 4.

| Site | Individuals excluded | Individuals remaining | Individuals marked (♂/♀) | Individuals recaptured (♂/♀) |
|------|----------------------|-----------------------|---------------------------|-----------------------------|
| 1    | 4                    | 41                    | 65/6                      | 10/0                        |
| 2    | 1                    | 10                    | 11/2                      | 0/0                         |
| 3    | 11                   | 50                    | 86/14                     | 25/0                        |
| 4a   | 1                    | 34                    | 119/11                    | 16/0                        |
| 4b   | 14                   | 42                    | —                         | —                           |
| 5    | 6                    | 43                    | 101/3                     | 23/0                        |
| 8    | 8                    | 0                     | 8/0                       | 0/0                         |
| 9    | 1                    | 41                    | 70/2                      | 5/0                         |
| 10   | 5                    | 0                     | 9/0                       | 0/0                         |
| Total| 37                   | 219                   | 469/38                    | 79/0                        |

The lowest value was chosen. Longevity was calculated based on the corrected Akaike information criterion (AICc), and survival was estimated from the data. Hence, data were split into two groups, based on whether the catching day was during the main flight season (June to mid-July) or not. Model selection was based on the corrected Akaike information criterion (AICc), and the lowest value was chosen. Longevity was calculated with \((−\ln(\varphi))^{-1}\). Since we could not gather any recatch at Sites 2, 8, and 10, they were excluded from analyses with MARK.

DNA extraction

DNA was extracted using QIAGEN DNeasy kit following the manufacturer’s instructions (with an extra 5-min incubation step for exuviae). All samples were genotyped at a panel of 10 (tibia samples) or 8 (exuviae) microsatellites loci using conditions described by Landmann et al. (2021).

Genotyping and sample selection

PCR was done in 5-µl reaction volumes, using the protocol described in Landmann et al. (2021). Fragments were analysed on a UnoCycler (VWR, Radnor, PA, USA). For improving the signal-to-noise ratio, all samples were cleaned using sephadex-columns prior to analysing to remove salts apparently introduced when using the buffer of the Q5 High Fidelity Polymerase (New England Biolabs, Frankfurt, Germany). LIZ500 was used as internal size standard. Fragment analysis was performed by commercial providers; in dealing with logistic issues due to an ongoing corona-virus pandemic, the provider had to be changed in the course of the project. Thus, the first seven loci (Cohy_4, Cohy_16, Cohy_19, Cohy_24, Cohy_33, Cohy_42, and Cohy_3) were genotyped by CRC Sequencing Facility (Chicago, IL, USA), who used an ABI 3130 instrument (Applied Biosystems, Foster City, CA, USA), and the remaining three loci (Cohy_43, Cohy_45, and Cohy_54) were genotyped by Eurofins (Hamburg, Germany), who likewise used an ABI 3130 instrument. Allele-scoring was performed with GeneMarker 3.0.1 (SoftGenetics, State College, PA, USA).

All samples produced results for at least one locus, but samples with a certain number of failures (i.e. null alleles) were excluded from each site to enhance genetic analyses (for details, see Table 1). We decided that at least 10 individuals were necessary for a (sub)population to be big enough to be included, and Sites 8 and 10 were thus excluded from further analyses (Table 1).

Population genetic analyses

The number of alleles (Na) and expected and observed heterozygosity (He, Ho) were calculated with GenALEx v6.5 (Peakall & Smouse, 2012), allelic richness (Ar), the number of private alleles (Nprivate), and Hardy–Weinberg tests (HWE) using the default settings of PopGenReport v3.0.4 (Adamack & Gruber, 2014), which was specifically developed for microsatellite data (Adamack & Gruber, 2014), in R v3.6.1 (R Core Team, 2019). Bonferroni–Holm correction was performed for all values. F-statistics were calculated using FSTAT v2.9.4 and hierfstat v0.5-7 (Goudet, 2003; Goudet & Jombart, 2020). Additionally, FreeNA (Chapuis & Estoup, 2007) was used to estimate (i) the frequency of null alleles over all loci and sites as well as per locus per site and (ii) Fst values corrected for null alleles using the ENA correction.

Pairwise linkage disequilibrium for all 10 loci was calculated using Genepop v4.7.5 (Rousset, 2008). Isolation by distance (IBD) was calculated with a Mantel test with 15 000 permutations, comparing geographic distance and Cavalli-Sforza and Edwards-Chord distance (Takezaki & Nei, 1996) using GenAlEx, poppr v2.6.6, ade4 v1.7-13 (Chessel et al., 2004; Kamvar et al., 2014) and visualised with MASS v7.3-51.4 (Venables & Ripley, 2002). Spearman rank order correlations were calculated to test the relationships of longevity with allelic richness (as one of the measures of genetic diversity with allelic richness (as one of the measures of genetic
diversity, Reed & Frankham, 2003, which should be less prone to null-allele issues than heterozygosity) and with Fis values.

To identify genetically distinct groups in the dataset, five approaches were chosen: (a) principal component analysis (PCA) as a widely used ordination method, without prior assumptions on the genetic groups; (b) discriminant analysis of principal components (DAPC) as a classification method that helps avoid that intra-group variability blurs the representation of among-group variability (an issue PCA has been criticised for, Jombart et al., 2010), assuming the populations sampled to constitute genetic groups; (c) DAPC without prior grouping, that is, exploring a range of different numbers of genetic groups; approaches (a)–(c) were calculated with adegenet v2.3.1 (Jombart, 2008). For (b), samples were separated into six geographic regions, each sampling site thus representing one region (see Fig. 1, for sample sizes see Table 1). Additionally, approach (b) was calculated using two groups based on their assignment to clusters of approach (d) for a number of clusters equalling 2. For (c), adegenet was used to calculate the number of potential clusters.

In addition, two model-based Bayesian cluster analyses were performed, one (approach d) without and one (e) with spatially explicit assumptions about the genetic groups present (see Fig. 3). Approach (d) was done using STRUCTURE v2.3 (Pritchard et al., 2000) for K = 1 to 10, 2 000 000 generations burn-in, 6 000 000 MCMC generations, and 10 replicates. Data visualisation and ΔK statistics (Evanno et al., 2005) were done with pophelper v 2.3.0 (Francis, 2017). Finally, (e) was calculated in Geneland v4.9.2 (Guillot et al., 2005a, 2005b, 2008, 2012; Guillot, 2008; Guillot & Santos, 2010, 2009; Guedj & Guillot, 2011) using the spatial model for K = 1 to 10, 500 000 generations burn-in and 5 000 000 MCMC generations.

A χ² test and a Mann–Whitney rank sum test were executed to test potential correlations of STRUCTURE-clusters with sex and with longevity.

Table 2. Estimates of population sizes and longevity of males of Coenagrion hylas at five sites in the Lech valley 2019. Model selection: Based on the lowest Akaike value, models were chosen to calculate population estimates and longevity for each site sampled in 2019. Given are the final selected model, population size including standard deviation, longevity for individuals in days, sample size of the capture-mark-recapture study (N_{total}), number of recaptured individuals on-site (r₁), and number of individuals recaptured at a different site (r₂). Parameters of selected models are: (i) φ₀, the probability of survival between two occasions; (ii) p₁, the catchability on day 1; (iii) pent, the probability of entering the population; and (iv) N, the total number of individuals. Since no female individuals could be recaptured, values present males only. Site 2 was excluded due to a lack of recaptures.

| Site | Model | Population size | Longevity | N_{total} | r₁ | r₂ |
|------|-------|----------------|-----------|-----------|----|----|
| 1    | φ₀(p)pent(g₄)N(g₄t) | 318 ± 125      | 7.3       | 65        | 8  | 2  |
| 3    | φ₀(p)pent(g₄t)N(g₄t) | 163 ± 66       | 9.6       | 93        | 22 | 3  |
| 4    | φ₀(p)pent(g₄t)N(g₄t) | 591 ± 161      | 5.4       | 120       | 12 | 3  |
| 5    | φ₀(p)pent(g₄t)N(g₄t) | 259 ± 55       | 4.6       | 106       | 21 | 2  |
| 9    | φ₀(p)pent(g₄t)N(g₄t) | 84 ± 8         | 3.6       | 70        | 3  | 2  |

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Table 3. Proven migration/dispersal distances of *Coenagrion hylas* by catch-recapture-methods in the Lech valley. Listed are sites of first capture and recapture as well as days between first capture and recapture (including day of first capture) as an indicator for minimum longevity and speed of dispersal. Besides the minimum air distance that must be travelled to travel from one site to another, the distance that must be passed by flying along the Lech is given.

| Site of first catch | Site of recatch | Days between first catch/recatch | Distance air (km) | Distance Lech (km) |
|---------------------|-----------------|---------------------------------|-------------------|-------------------|
| 1                   | 4               | 29                              | 8.98              | 12.5              |
| 1                   | 5               | 19                              | 16.7              | 21.3              |
| 3                   | 5               | 8                               | 0.26              | 0.39              |
| 3                   | 5               | 0                               | 0.35              | 0.48              |
| 3                   | 5               | 22                              | 0.27              | 0.40              |
| 5                   | 3               | 6                               | 0.29              | 0.40              |
| 5                   | 3               | 15                              | 0.30              | 0.45              |
| 4                   | 5               | 9                               | 8.02              | 10.3              |
| 4                   | 3               | 4                               | 8.40              | 10.5              |
| 4                   | 3               | 6                               | 8.42              | 10.6              |
| 9                   | 3               | 19                              | 11.3              | 14.5              |
| 9                   | 3               | 4                               | 11.4              | 14.7              |

Table 4. Genetic diversity measures for populations at each sampling site of *Coenagrion hylas*: observed (Ho) and expected (He) heterozygosity, allelic richness (Ar), number of private alleles (normalised to a sample of 20 individuals) (Nprivate), inbreeding coefficient (Fis) and P-values for test of Hardy–Weinberg equilibrium are given. Row 4a refers to tibiae, 4b to exuviae of Site 4. Asterisks indicate significance at different confidence levels after Bonferroni–Holm correction: *Confidence at a level of 95%; **Confidence at a level of 99%; ***Confidence at a level of 99.9%.

| Site | Ho   | He   | Ar   | Nprivate | Fis | HWE test |
|------|------|------|------|----------|-----|----------|
| 1    | 0.532| 0.592| 4.999| 0.300    | 0.103***| 0.091    |
| 2    | 0.623| 0.523| 4.429| 0.300    | −0.191 | 0.241    |
| 3    | 0.547| 0.589| 4.881| 0.700    | 0.070***| 0.060    |
| 4a   | 0.532| 0.556| 4.885| 0.200    | 0.042***| 0.010    |
| 4b   | 0.525| 0.559| 4.209| —        | 0.090***| 0.040    |
| 5    | 0.523| 0.558| 5.007| 0.400    | 0.063***| 0.006*   |
| 9    | 0.492| 0.543| 4.678| 0.400    | 0.094** | 0.108    |

Table 5. Pairwise Fst-values (calculated after Nei 1987) for six subpopulations of *C. hylas* at sites in the Lech valley. Asterisks indicate significance at different confidence levels: *Confidence at a level of 95%; **Confidence at a level of 99%; ***Confidence at a level of 99.9%.

| Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
|--------|--------|--------|--------|--------|
| Site 1 | —      | —      | —      | —      |
| Site 2 | 0.009  | —      | —      | —      |
| Site 4 | 0.025***| 0.015  | —      | —      |
| Site 3 | 0.005  | 0.009  | 0.025***| —      |
| Site 5 | 0.009* | 0.003  | 0.031** | 0.007  |
| Site 9 | 0.010***| 0.006  | 0.027* | 0.005  | 0.003  |

population model output for all models tested and compared in MARK for the AICc is given in Supplementary Table 1.

**Connectivity**

For individuals recaptured at their site of origin, the average distance between first capture and recapture was 36 m. We also gathered recaptures that prove migration over distances greater than 8 km along the centre line of the Lech valley (see Table 3; Fig. 1). In at least four cases, the Lech was crossed, and the river therefore was no insurmountable barrier for *C. hylas*.

**Impact of tibia cutting**

Since *C. hylas* is a protected species, we tested whether taking genetic samples by cutting off tibiae reduced the chance of survival in marked individuals. Recapture rates from individuals of which tibiae had been collected (13.3%, *n* = 222) compared with those marked individuals of which no tissue had been collected (17.1%, *n* = 285) did not differ significantly (*χ²* = 0.095; *P* = 0.76).

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95% confidence interval) and differentiation for pairs of populations were low (Table 5). Estimation of null-allele frequency over all loci and sites was 0.065; the values per locus per site ranged from 0.000 to 0.267 (Supplementary Table 4). Pairwise $F_{st}$ values corrected for null alleles were, as a tendency, higher than the uncorrected ones, ranging from $-0.001$ to 0.087 (Supplementary Table 5). No significant correlation was found between longevity and allelic richness ($P = 0.950$) and longevity and $F_{is}$ values ($P = 0.950$).

No statistically significant IBD patterns were found (Mantel test, $r = -0.008, P = 0.624$; Supplementary Fig. 1) in the range of $C. hylas$ with genetic distances being quite variable within one geographic region but not among regions. The PCA showed no clear separation of sites and, instead, formed overlapping clusters. Also, axis loads were low and therefore explained just little of the variation in the data. In contrast, a DAPC using the sample sites as prior assumption, revealed weak separation of sites, with Site 4 differing the most from other sites (Supplementary Fig. 2), which can also partly be seen in some of the genetic diversity values (Tables 4 and 5). The DAPC analyses without using prior groupings resulted in a number of seven groups best representing the data (Supplementary Fig. 2).

When doing Bayesian clustering with STRUCTURE, the method introduced by Evanno et al. (2005) resulted in $K = 2$ to be the best value (Supplementary Fig. 3). Two distinct clusters were seen within each population, but there was no clear separation between sites (Fig. 3). Also, when looking at $K = 3$ and $K = 4$, the same pattern merged (Fig. 3). Neither sex ($P = 0.156$) nor longevity ($P = 0.456$) were statistically significant indicators for the differences between the clusters proposed by STRUCTURE. Finally, the Bayesian clustering method as implemented in Geneland returned $K = 1$ as the most likely result (Fig. 3).

Discussion

Studies combining direct observations of dispersal (i.e. by capture-mark-recapture) with indirect methods (i.e. gene-flow estimates) are still rare for insects, and evidence for a close correlation between the results of these approaches in odonates is conflicted (Watts et al., 2004, 2007a, 2007b; Keller et al., 2010; Hassall & Thompson, 2012; Keller & Holderegger, 2013). In our study, mark-recapture data and the population genetic analysis revealed congruent results. The ecological approach showed that males of $C. hylas$ exhibit pronounced dispersal abilities, enabling them to cover distances of more than a dozen kilometres in comparatively short time and to cross barriers and unsuitable habitats between sites of occurrence which are isolated from each other. This high mobility was reflected in the genetic structure of the investigated subpopulations which are scattered along 30 km of the Lech river valley. We observed a well-mixed genetic structure within all investigated sites and did not detect signs of IBD. Also, the results of Bayesian clustering methods are in line with a near-panmictic character of the Lech valley populations.

Population history

After its eradication at the first known European site of appearance in Germany (Bilek, 1954), the rediscovery of $C. hylas$ at a mountain lake in Tyrol back in 1974 was quite an entomological sensation (Heidemann, 1974). This specific lake, from where the species vanished decades ago, is in about 17.5 km air distance southeast from Site 1 but is separated from it by high mountain chains. Following water courses, the
distance to the lower Lech valley from the lake to Site 1, where the species has been known to be present since 1986, is about 30 km. Based on the topographical setting, one could expect that *C. hylas* has successively spread over the Lech river valley from a founder population at Site 1 and that genetic distance between sites would increase with distance from Site 1.

Our genetic data do not support such a scenario: Genetic differentiation showed no gradient upstream towards Site 9, which is most distant from Site 1. There was no clear geographic pattern of differentiation; only Site 4 showed signs of stronger differentiation from the other sites. Pairwise $F_{st}$-values were very low and mostly not significantly different from zero; the corresponding values corrected for null alleles were higher (Supplementary Table 5) but the general trend of ample gene flow across sites was nevertheless corroborated. Also, the number of private alleles was very low and thus does not reveal a situation in which subpopulations are genetically highly different from each other (see the ‘Genetic diversity and inbreeding’ section). It therefore seems likely that the upstream populations at Sites 2–9, which had not been detected until between 2000 and 2005 (Müller, 2001, 2015) may have been overlooked in the 20th century. Therefore, Sites 2–9 may either originate from one single invasion event decades ago and/or the time elapsed since the founding events (in combination with incomplete isolation due to the dispersal ability of the species) are too short to foster a clear genetic differentiation.

We note that two technical aspects will need additional attention in future projects on these populations. Firstly, the thresholds we used for excluding individuals in dealing with genotyping failure differed across sites, which may have resulted in differences how the sites are genetically represented in the data set, albeit to unknown extent. Secondly, for the remaining individuals, null alleles were identified, which affected $F_{st}$ calculations slightly. For avoiding or at least dealing with these two issues, more individuals should be analysed, and the same individuals should be genotyped multiply. At the same time, we note the general soundness of the body of data acquired using multiple approaches, which all point in the same direction.

**Demographics**

Capture-mark-recapture/resight studies tend to yield rather high recapture/resight rates of 30 to 80% in adult male damselflies (for overview, see Allen & Thompson, 2010; for coenagrionids, see Beirinckx *et al.*, 2006; Sherratt *et al.*, 2010; Hassall & Thompson, 2012; Keller & Holderegger, 2013). Compared with similar studies, our overall recapture rate of 16.8% for males is quite low and does not exceed 20% even if we exclude males which have been first captured late in the season and at sites where no recaptures were achieved. In our opinion, these low recapture rates could already be a hint for high mobility (see also Müller, 2000, unpubl. thesis, Univ. Ulm) and low philopatry in *C. hylas*, in that high levels of philopatry should foster high recapture rates (Hassall & Thompson, 2012). Given the large geographic extent of our study area and assuming a high portion of dispersive males in the investigated subpopulations, comparatively low recatch rates are not surprising. In return, the percentage of recatches at sites other than the marking site (15%) is rather high. Also, *C. hylas* has very specific demands with regards to sunshine (Ott, 2003). As soon as it is a bit cloudy, all individuals vanish into close wood patches and are nearly impossible to be encountered, which likely also influenced the chances for recaptures.

Based on hatching and time of flight, Müller (2000) already suggested a maximum longevity of 40 days for certain individuals of *C. hylas*. Our data support this assumption, although 50% of all males were recaptured not later than 10 days after their first catch, and calculations with MARK resulted in an average longevity of about 12 days. However, more than a third of all individuals were recaptured after more than 2 weeks, and 15% were at least 3 weeks old when recaptured. These results are above average for damselfly males (i.e. average longevity 7–8 days, maximum 29 days for *Coenagrion mercuriale*, Rouquette & Thompson, 2007, or 5.7 average and 19 days maximum for *Coenagrion ornatum*; Körner *et al.*, 2018 and pers. comm.) and are possibly even higher for females, which tend to live longer in damselflies (Cordero, 1994).

**Dispersal and connectivity**

During dispersal, many dragonfly species tend to use habitat types that are also preferred for reproduction (stepping-stone hypotheses). Hence, the knowledge of dispersal possibilities and dispersal behaviour is essential for evaluating a species’ connectivity (Rouquette & Thompson, 2007). It is known that the extent of dispersal in odonates depends on various factors, including sex (Beirinckx *et al.*, 2006), body size (Anholt, 1990; Thompson, 1991), and age (Conrad *et al.*, 2002). In addition, environmental conditions and population densities, which can vary from year to year, may also impact movement patterns. However, although our study is only based on a single year of data collection, such potential variations are not important here because our main concern is to demonstrate the dimension of the dispersal potential in *C. hylas* in connection with its impacts on genetic isolation patterns and nature conservation measures, regardless of possible year-to-year variations.

In general, lotic species tend to have lower dispersal abilities than lentic species (Hof *et al.*, 2006; Grewe *et al.*, 2013). This could explain that lotic species have, on average, smaller ranges, partly because they (like *C. hylas*) are mostly habitat specialists and face problems in colonising appropriate habitats due to the lack of stepping-stone habitats in the interjacent areas (Kaakman *et al.*, 2018). Additionally, wing aspect ratio has a significant effect on species’ range sizes in coenagrionids (Swaegers *et al.*, 2014). With *C. hylas* being the bulkiest of all coenagrionids and having exceptionally high wing aspect ratios (Swaegers *et al.*, 2014), this would already suggest higher dispersal rates for the species. In fact, our data indicate a much higher dispersal ability and dispersal distances for *C. hylas* than in other damselflies and especially other coenagrionid species.

A recent study in Styria, Austria (Körner *et al.*, 2018) resulted in maximum dispersal distances of 300 m for *C. ornatum*. For *C. mercuriale*, various studies resulted in dispersal distances of only 500 m up to a maximum of 2 km (Rouquette & Thompson, 2007; Hassall & Thompson, 2012; Keller &
Even for the bigger demoiselles *Calopteryx splendens* and *Calopteryx virgo* and for small- to medium-sized dragonflies like *Leucorrhinia caudalis*, the maximum radius of action is considered to be 4 (Stettmer, 1996) and 5 km (Keller et al., 2010), respectively. In line with this, at an average of 36 m, we also recorded most recaptures within the proximity of the first capture. However, especially at larger study sites (Site 1, Site 4), we recorded higher ranges with distances travelled between sections of the water bodies and to adjoining hunting and resting areas of up to 300 m (see Müller, 2000 for similar results). In seven cases (9% of all recaptures), we witnessed dispersal over distances greater than 5 and even up to 20 km along the centre line of the valley.

Especially for rheophile species, dispersal is assumed to happen along water streams (Stettmer, 1996; Purse et al., 2003; Körner et al., 2018). Considering individuals would have had to pass high mountain ridges for travelling along more direct routes, we believe this is also true for *C. hylas*, meaning that the actual travelling distances between sites likely are even longer. Of special notice are four incidents of individuals that must have crossed the river Lech (Site 1 → Site 5, Site 4 → Site 3/Site 5), meaning that the Lech does not represent an insurmountable barrier for these damselflies. Still, it must be considered that all recaptures on the other side of the river have been close to a spot where the river is just 25 m wide, whereas river width is 100–250 m in other segments.

We only recaptured male individuals, which is not uncommon in damselflies (see for instance Conrad et al., 2002; La Porta & Goretti, 2019), but there are signs that female zygopterans might be dispersing even further (Beirinckx et al., 2006). Colonisation of new sites cannot be achieved without oviposition done by females, and so we assume that dispersal rates and abilities in female *C. hylas* must be at least comparable with those in males.

Good dispersal capability is also in line with the molecular-genetic results. IBD calculations indicate high connectivity, showing no significant patterns between sites but high variation within sites. This is also reflected by the combined evidence from using five approaches to identifying distinct genetic groups in the data. In combining the results from these five approaches, the peculiarities of the methods used under various population–genetic scenarios need be considered. PCA barely revealed structure, which, in itself, is difficult to interpret given that weak population structure and a difficult ratio of within- to among-group variability cannot be distinguished (Jombart et al., 2010). DAPC returned weak separation of sites, when the six geographic regions sampled were used as an assumption, and a number of seven genetic groups when no prior grouping was used. In interpreting the results from DAPC used in the two modes, we take into account the caveat by Cullingham et al. (2020) that DAPC can produce imprecise results under strong migration and resulting low Fst (and Fst stays low for our sites also after correction for null alleles), in particular when combined with smaller census sizes. We thus consider the results of very low numbers for K by STRUCTURE and Geneland as biologically more plausible (see next paragraph about the issue of K = 2 as a result from STRUCTURE). In essence, we infer that there is just slight differentiation of populations and that populations are well connected.

The results of K = 2 by STRUCTURE deserve separate reflection. For clustering analyses with K = 2, the real number of clusters could always be K = 1 (Evanno et al., 2005). If this would be the case, each individual should show a balanced allocation to both clusters. Even though clusters are not showing separation among sites, there are always two distinct clusters to be seen within each site. No biological factor that would account for this pattern could be validated statistically. A recent study covered the infection rate of *Wolbachia* in damselflies from coenagrionids (Lorenzo-Carballa et al., 2019). Although this study was done in a different latitude, an infection with, for example, two incompatible *Wolbachia* strains could be a possible explanation for the separation of individuals within a site, since an infection with *Wolbachia* can trigger reproductive incompatibility of individuals of different infection status (Werren et al., 2008).

Whether a hypothetical *Wolbachia*-based differentiation into two genetic groups despite ample gene flow across sites is likely will need separate analysis. For the time being, it may be more robust to assume that the real number of K equals 1, as returned by Geneland, in particular given the high frequency of K = 2 by STRUCTURE later identified as spurious (Janes et al., 2017), especially when strong migration applies (Cullingham et al., 2020).

Generally, quantifying the relative extent of migration in the various directions possible across sites can be of direct relevance to conservation strategies. Here, however, we have refrained from doing so due to uneven and overall small population sizes, both hampering interpretation of the results of such a calculation (Sundqvist et al., 2016). Moreover, the demonstrated effect of null alleles on calculating population differentiation would also affect this calculation, without a possibility of correcting for it.

**Genetic diversity and inbreeding**

If dispersal results in successful reproduction at a new site, dispersal translates into gene flow (Keller et al., 2010 after Allendorf & Luikart, 2007). Therefore, expecting our field results to be similar to molecular methods, subpopulations should show the same signs of genetic diversity. Since inbreeding is less frequent in bigger habitat networks than in smaller ones (Nonaka et al., 2019) and dispersal suggests a connected meta-population network in the Lech valley, we consider a very low risk of current inbreeding depression at any site. Inbreeding coefficients were significantly elevated for five sites, but they do not seem eminently high compared with levels reported from similar studies (Keller et al., 2012; Herzog & Hadrys, 2017). In addition, Fis-values could be related to population sizes, since bigger populations have less signs of inbreeding.

In zygopterans, different levels of genetic diversity can appear when using microsatellite loci (Watts, 2009), and at a range margin, species with low dispersal ability like *C. mercuriale* may be almost monomorphic at microsatellite loci (see Watts et al., 2005a). However, our data of the expected heterozygosity are placed well on average levels (Watts, 2009). Heterozygote deficits are quite common for odonate microsatellite loci, and this phenomenon is thought to be related to technical problems such as null alleles in addition to or even rather than to biological factors like population subdivision and assortative mating (Watts, 2009). The estimation of null-allele frequencies confirms...
that null alleles are included (Supplementary Table 4). For Site 2, only 10 individuals were genotyped (compared to more than 30 for all other sites), and heterozygosity results thus could be coincidental and an artefact of sampling rather than actual results. However, allelic richness, less sensitive to null-allele issues than heterozygosity, was similar for all sites, with slightly lower results for exuviae. Its values were average or slightly elevated compared with studies with the same magnitude of standardised samples sizes: around 3.0 based on 18 individuals for *Erythromma viridulum* (Watts et al., 2010), 2.4–3.5 based on 19 individuals for *C. mercuriale* (Lorenzo-Carballa et al., 2015), and 5.1–5.4 for *Orthehtrum coerulescens* based on 12 individuals (Herzog & Hadrys, 2017). Species like *Nehalennia speciosa*, which show signs of genetic poverty, have an allelic richness of around 1.2 (based on 30 individuals; Bernard & Schmitt, 2020).

**Methodological issues: sampling exuviae**

When collecting DNA samples of endangered species, researchers always have to balance minimally invasive methods with methods that produce enough DNA yield (see Watts et al., 2005b, Keller et al., 2010, for odonates; Oi et al., 2013 for bees; Suzuki et al., 2012 for beetles). Whilst we could prove that sampling of mid-leg tibiae did not harm individuals in a way that would reduce their survival, it was still important to us to check whether exuvial DNA would be sufficient for genetic methods. Non-lethal tissue sampling in insects has shown to be quite difficult, since faeces and exuviae usually do not convey enough material for successful amplification (Watts et al., 2005b; Oi et al., 2013). Using exuviae for genetic measurements and species identification has of course been done before, not only for odonates (Monroe et al., 2010) but also for other arthropods like tarantulas (Petersen et al., 2007). On the one hand, exuviae have been stated to be a reliable source of DNA (Watts et al., 2005b) but on the other hand have proven to be quite problematic as genetic sample (Keller et al., 2010). For odonates, in which exuviae are shed by aquatic larvae, there are even some more difficulties, especially concerning the sampling procedure. DNA degradation occurs fast if samples are exposed to sunlight or hydrated (Watts et al., 2005b). For sample collection, this means that exuviae are best collected when extremely fresh. Another important factor may be proper storing of samples until extraction. We used a dry shipper filled with liquid nitrogen to store samples until transfer into a freezer, but no ethanol was used as this is known to increase sample fragility (Ozana et al., 2020). Moreover, collection was only done in the early morning under good weather conditions, when exuviae were likely to be fresh. Our amplified results showed no deficiency compared with DNA collected from mid-legs, which supports that our stringent sampling and storing procedure for exuviae may be relevant. Overall, we proved that using exuvial DNA for genetic methods in *C. hylas* can be successful when stored cautiously.

**Conclusions and implications for conservation**

The species’ dispersal ability is considerable, but the rarity and the extremely restricted range of *C. hylas* in Central Europe hint at very specific habitat demands of the species. Therefore, we can support the above-mentioned notion of Kalkman et al. (2018) that especially lotic species in fragmented landscapes need stepping-stone habitats to colonise appropriate habitats.

Currently, the Lech valley (sub)populations of *C. hylas* seem to be sufficiently connected throughout the valley and genetically healthy with no signs of inbreeding. However, with only a few very small sites occupied and with increasing pressure on all water bodies due to the touristic development of the Natura 2000 area, the local population is still highly vulnerable. In addition, climate warming also causes problems. In 2019, a few small water bodies where the species was reproducing until the first decade of the 21st century were completely desiccated and no longer suitable for the species. Given the clear signs of widespread dispersal throughout the mountain valley, management measurements should include the creation of stepping stones and potential reproduction habitats in-between existing sites to possibly enhance the species’ habitats.

Our data confirm that DNA can successfully be extracted from exuviae and thus can be used for population-genetic studies rather than gaining DNA from living individuals, which always is potentially problematic in an endangered species. As, in addition, exuviae are also a very important tool in monitoring endangered species (Foster & Soluk, 2004) and are essential when it comes to avoiding sampling biases (Raebel et al., 2010), using exuviae as a general tool when assessing the state of *C. hylas* might be a good approach in the future.

We conclude that future monitoring in the valley should be done on a more regular basis to keep an eye on the European main stronghold of this endangered species and to check on the viability of these subpopulations. In addition, a specific follow-up study should be designed with emphasis to clarify possible source–sink structures within the network of populations in the valley, which could possibly facilitate to focus conservation measures on the most important sites.

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Data availability statement

Data available in article supplementary material.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Figure 1: Isolation by distance using Cavalli-Sforza and Edwards Chord distance for subpopulations of Coenagrion hylas in the Lech valley. Mantel test showed no significance for these patterns (p > 0.05).

Supplementary Figure 2: (A) Principal Component Analysis, (B) Discriminant Analysis of Principal Components (DAPC) of Coenagrion hylas microsatellite data with prior grouping, and (C) Discriminant Analysis of Principal Components (DAPC) of Coenagrion hylas microsatellite data without using prior groupings.

Supplementary Figure 3: Statistics on the results of STRUCTURE analysis for Coenagrion hylas in the Lech valley, calculated after Evanno et al. (2005). (A) mean likelihood across replicates +/- SD, (B) first derivative of likelihood across replicates +/- SD, (C) second derivative of likelihood across replicates +/- SD, (D) delta K dependent on the value of K.

Supplementary Table 1: Total population model output for all models tested and compared in MARK for the AICc (corrected Akaike-criterion) calculated by the programme. Number of parameters (n p) used for calculating this models and deviance corrected Akaike-criterion calculated by the programme. Number of replicates

Supplementary Table 2: Results for the corresponding Hardy–Weinberg-tests for all loci and sites. Values were calculated using the package pegas in R v3.6.1. Asterisks indicate significance after Bonferroni-Holm-correction.

Supplementary Table 3: Pairwise linkage disequilibrium for all ten loci calculated using Genepop v4.7.5. After Bonferroni-Holm-correction no significance between a pair of loci could be detected at a 95% confidence level.

Supplementary Table 4: Pairwise Fst-values (calculated after Weir, 1996) for all six subpopulations. The table shows each pair of populations with the use of ENA correction described in Chapuis and Estoup (2007). Asterisks indicate significance at a confidence level of 95%.

Supplementary Table 5: Estimation of the null allele frequency using the EM algorithm (Dempster et al. 1977) with the software FreeNA. For all locus x population-combinations the frequency is given. Overall null allele frequency for all loci was 0.065.

References

Adamack, A.T. & Gruber, B. (2014) PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution, 5(4), 384–387. https://doi.org/10.1111/2041-210X.12158.

Allen, K.A. & Thompson, D.J. (2010) Movement characteristics of the Scarce Blue-tailed Damselfly, Ischnura pumilio. Insect Conservation and Diversity, 3(1), 5–14. https://doi.org/10.1111/j.1752-4598.2009.00070.x.

Allendorf, F.W. & Luikart, G. (2007) Conservation and the genetics of populations, 2nd Edn. Blackwell, Malden, Massachusetts.

Alp, M., Keller, I., Westram, A.M. & Robinson, C.T. (2012) How river structure and biological traits influence gene flow: a population genetic study of two stream invertebrates with differing dispersal abilities. Freshwater Biology, 57(5), 969–981. https://doi.org/10.1111/j.1365-2427.2012.02758.x.

Anholt, B.R. (1990) Size-biased dispersal prior to breeding in a damselfly. Oecologia, 83(3), 385–387. https://doi.org/10.1007/BF00317564.

Beirinckx, K., Van Gossum, L., Lajeunesse, M.J. & Forbes, M.R. (2006) Sex bias in dispersal and philopatry: insights from a meta-analysis based on capture-mark-recapture studies of damselflies. Oikos, 113(3), 539–547. https://doi.org/10.1111/j.2006.0030-1299.14391.x.

Bernard, R. & Daraž, B. (2010) Recull occurrence of east palaearctic dragonflies in northern European Russia, with first records of Coenagrion glaciale in Europe (Odonata: Coenagrionidae). International Journal of Odonatology, 13(1), 39–62. https://doi.org/10.1080/13887890.2010.9748359.

Bernard, R. & Schnitt, T. (2020) Genetic poverty of an extremely specialized wetland species, Nehalennia speciosa: implications for conservation (Odonata: Coenagrionidae). Bulletin of Entomological Research, 100, 405–413. https://doi.org/10.1017/S0007485309990381.

Bilek, A. (1954) Eine neue Agrionide aus Bayern (Odonata). Nachrichtenblatt der Bayerischen Entomologen, 3, 97–99. http://www.biodiversitylibrary.org/.

Chapuis, M.P. & Estoup, A. (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution, 24(3), 621–631.

Chessel, D., Dufour, A.B., & Thioulouse, J. (2004) The ade4 package I: one-table methods (Vol. 1, 1). http://pbil.univ-lyon1.fr/

Conrad, K.F., Willson, K.H., Whitmeyer, R., Thomas, C.J. & Sherratt, T.N. (2002) Characteristics of dispersing Ischnura elegans and Coenagrion puella (Odonata): age, sex, size, morph and ectoparasitism. Ecography, 25(4), 439–445. https://doi.org/10.1034/j.1600-0587.2002.250406.x.

Cordero, A. (1994) The effect of sex and age on survivorship of adult damsels in the laboratory (Zygoptera: Coenagrionidae). Odonatologica, 23(1), 1–12.

Cullingham, C.I., Miller, J.M., Peery, R.M., Dupuis, J.R., Malenfant, R.M., Gorrell, J.C. & Janes, J.K. (2020) Confidently identifying the correct K value using the ΔK method: When does K = 2? Molecular Ecology, 29, 862–869. https://doi.org/10.1111/mec.15374.

Cullingham C.I., Miller J.M., Peery R.M., Dupuis J.R., Malenfant R.M., Gorrell J.C., & Janes J.K. (2020) Confidently identifying the correct K value using the ΔK method: When does K = 2?: Molecular Ecology, 29(5), 862–869. https://doi.org/10.1111/mec.15374.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I.,Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., & Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. In Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I.,Sherratt, T.N. (2002) Characteristics of dispersing

© 2021 The Authors. Insect Conservation and Diversity published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. Insect Conservation and Diversity, doi: 10.1111/icad.12516
Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Foster, S.E. & Soluk, D.A. (2004) Evaluating exuvia collection as a management tool for the federally endangered Hine’s emerald dragonfly, *Somatochlora hineauna* Williamson (Odonata: Corduliidae). *Biological Conservation, 118*(1), 15–20. https://doi.org/10.1016/j.biocon.2003.06.002.

Francis, R.M. (2017) pophelp: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources, 17*(1), 27–32. https://doi.org/10.1111/1755-0998.12509.

Guillot, G., Santos, F. & Estoup, A. (2008) Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. <http://www2.unil.ch/popgen/softwares/fstat.htm>.

Goudet, J., & Jombart, T. (2020)

Hof, C., Brandle, M. & Brandle, R. (2006) Lentic odonates have larger and more northern ranges than lotic species. *Journal of Biogeography, 33*(1), 63–70. https://doi.org/10.1111/j.1365-2699.2005.01358.x.

Hughes, J.M., Schmidt, D.J. & Finn, D.S. (2009) Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. *Biotechnology, 59*(7), 573–583. https://doi.org/10.1052/bio.2009.57.7.

Jones, J.K., Miller, J.M., Dupuis, J.R., Malenfant, R.M., Gorrell, J.C., Cullingham, C.I. & Andrew, R.L. (2017) The K = 2 conundrum. *Molecular Ecology, 26*, 3594–3602. https://doi.org/10.1111/mec.14187.

Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Jombart, T., Devillard, S. & Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics, 11*, 94. https://doi.org/10.1186/1471-2156-11-94.

Kalkman, V.J., Boudot, J.-P., Bernard, R., Conze, K.-J., De Knijf, G., Dytatova, E., Ferreira, S., Jović, M., Ott, J., Riservato, E. & Sahlién, G. (2010) European Red List of Dragonflies. Publications Office of the European Union, Luxembourg.

Kalkman, V.J., Boudot, J.-P., Rafal, B., De Knijf, G., Suhling, F. & Termaat, T. (2018) Diversity and conservation of European dragonflies and damselflies (Odonata). *Hydrobiologia, 811*, 269–282. https://doi.org/10.1007/s10750-017-3495-6.

Kamvar, Z.N., Tabima, J.F. & Grünewald, N.J. (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ, 2014*(1), 1–14. https://doi.org/10.7717/peerj.281.

Keller, D., Brodbeck, S., Flöss, I., Vonwül, G. & Holderegger, R. (2010) Ecological and genetic measurements of dispersal in a threatened dragonfly. *Biological Conservation, 143*(11), 2658–2663. https://doi.org/10.1016/j.biocon.2010.07.008.

Keller, D. & Holderegger, R. (2013) Damselflies use different movement strategies for short- and long-distance dispersal. *Insect Conservation and Diversity, 6*(5), 590–597. https://doi.org/10.1111/icad.12016.

Keller, D., Van Strien, M.J. & Holderegger, R. (2012) Do landscape barriers affect functional connectivity of populations of an endangered damselfly? *Freshwater Biology, 57*(7), 1373–1384. https://doi.org/10.1111/j.1365-2427.2012.02797.x.

Körner, A., Rieckh, C. & Holzinger, W.E. (2018) Die Vogelazurjungfer (Coenagrion ornatum) am Laabach (Grazier Feld, Steiermark): Beobachtung und Biologie einer EU-geschützten Libellenart. *Entomologica Austriaca, 25*, 145.

La Porta, G. & Goretti, E. (2019) Investigation on the declining southern damselfly (Coenagrion mercuriale, Odonata) in a Mediterranean population: survival rate and population size. *Journal of Insect Conservation, 23*(4), 667–675. https://doi.org/10.1007/s10841-019-00160-y.

Landmann, A. (2013) Siberia in the Alps: recent status, habitat requirements, and conservation of Coenagrion hylas in Central Europe. *International Congress of Odonatology, Freising, Bavaria, Germany - Book of Abstracts, 3*.

Landmann, A., Lehmann, G., Mungenast, F. & Sonntag, H. (2005) Die Libellen Tirols. Watts, Austria: Berenkamp.

Landmann, M., Schilling, M., Landmann, A., Stein, F.M. & Schlick-Steiner, B.C. (2021) Isolation and characterization of 10 polymorphic microsatellite loci in the rarest European damselfly, *Coenagrion hylas* (Odonata: Coenagrionidae). *International Journal of Odonatology* accepted.

Lebret, J.-D., Burnham, K.P., Clobert, J. & Anderson, D.R. (1992) Modeling survival and testing biological hypotheses using marked
Thompson, D.J. (1991) Size-biased dispersal prior to breeding in a damselfly: conflicting evidence from a natural population. *Oecologia*, 87(4), 600–601. https://doi.org/10.1007/BF00320427.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*, 4th Edn. New York, NY, USA: Springer. http://www.stats.ox.ac.uk/pub/MASS4/.

Wasser, P.M., Austad, S.N. & Keane, B. (1986) When should animals tolerate inbreeding? *The American Naturalist*, 128(4), 529–537. https://www.jstor.org/stable/2461335?seq=1#metadata_info_tab_contents.

Watts, P.C. (2009) Characteristics of microsatellite loci in Odonata. *International Journal of Odonatology*, 12(2), 275–286. https://doi.org/10.1080/13887890.2009.9748345.

Watts, P.C., Keat, S. & Thompson, D.J. (2010) Patterns of spatial genetic structure and diversity at the onset of a rapid range expansion: colonisation of the UK by the small red-eyed damselfly *Erythromma viridulum*. *Biological Invasions*, 12, 3887–3903. https://doi.org/10.1007/s10530-010-9779-7.

Watts, P.C., Rouquette, J.R., Saccheri, I.J., Kemp, S.J. & Thompson, D.J. (2004) Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion mercuriale*. *Molecular Ecology*, 13(10), 2931–2945. https://doi.org/10.1111/j.1365-294X.2004.02300.x.

Watts, P.C., Saccheri, I.J., Leblois, R., Kemp, S.J. & Thompson, D.J. (2007a) Compatible genetic and ecological estimates of dispersal rates in insect (*Coenagrion mercuriale*: Odonata: Zygoptera) populations: analysis of “neighbourhood size” using a more precise estimator. *Molecular Ecology*, 16(4), 737–751. https://doi.org/10.1111/j.1365-294X.2006.03184.x.

Watts, P.C., Saccheri, I.J., Kemp, S.J. & Thompson, D.J. (2005a) Population structure and the impact of regional and local habitat isolation upon levels of genetic diversity of the endangered damselfly *Coenagrion mercuriale* (Odonata: Zygoptera). *Freshwater Biology*, 51(2), 1365–2477. https://doi.org/10.1111/j.1365-2427.2005.01478.x.

Watts, P.C., Saccheri, I.J., Kemp, S.J. & Thompson, D.J. (2007b) Effective population sizes and migration rates in fragmented populations of an endangered insect (*Coenagrion mercuriale*: Odonata). *Journal Animal Ecology*, 76(4), 790–800. https://doi.org/10.1111/j.1365-2656.2007.01249.x.

Watts, P.C., Thompson, D.J., Daguett, C. & Kemp, S.J. (2005b) Exuviae as a reliable source of DNA for population-genetic analysis of odonates. *Odontologica*, 34(2), 183–187.

Werren, J.H., Baldo, L. & Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, 6(10), 741–751. https://doi.org/10.1038/nrmicro1969.

White, G.C. & Burnham, K.P. (1999) Program mark: survival estimation from populations of marked animals. *Bird Study*, 46, S120–S139. https://doi.org/10.1080/000635659909477239.

Wildermuth, H., & Küry, D. (2009) *Libellen schützen, Libellen fördern. Leitfaden für die Naturschutzpraxis. Beiträge zum Naturschutz in der Schweiz*. 31, 1–88.

Wildermuth, H. & Martens, A. (2019) *Die Libellen Europas*. Quelle&Mayer, Wiebelsheim, Germany.

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