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Differentiation of movement behaviour in an adaptively diverging salamander population

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Abstract

Dispersal is considered to be a species-specific trait, but intraspecific variation can be high. However, when and how this complex trait starts to differentiate during the divergence of species/lineages is unknown. Here, we studied the differentiation of movement behaviour in a large salamander population (*Salamandra salamandra*), in which individual adaptations to different habitat conditions drive the genetic divergence of this population into two subpopulations. In this system, salamanders have adapted to the deposition and development of their larvae in ephemeral ponds vs. small first-order streams. In general, the pond habitat is characterized as a spatially and temporally highly unpredictable habitat, while streams provide more stable and predictable conditions for the development of larvae. We analysed the fine-scale genetic distribution of larvae, and explored whether the adaptation to different larval habitat conditions has in turn also affected dispersal strategies and home range size of adult salamanders. Based on the genetic assignment of adult individuals to their respective larval habitat type, we show that pond-adapted salamanders occupied larger home ranges, displayed long-distance dispersal and had a higher variability of movement types than the stream-adapted individuals. We argue that the differentiation of phenotypically plastic traits such as dispersal and movement characteristics can be a crucial component in the course of adaptation to new habitat conditions, thereby promoting the genetic divergence of populations.

KEYWORDS
capture–mark–recapture, ecological speciation, microsatellite loci analysis, multistate mark–recapture model, passive implantable transponder tags, phenotypic plasticity, radio-tracking

1 | INTRODUCTION

Dispersal can be defined as individual movements during different life cycle stages across space (e.g., natal dispersal or breeding dispersal), with potential consequences for gene flow. Dispersal is a major life history trait that—by changing the composition of individuals in populations—directly or indirectly affects the ecology and evolution of a species (Ronce, 2007). As environmental conditions constantly change on a temporal and spatial scale, the persistence of populations strongly depends on the ability of individuals to disperse and exploit spatially and temporally variable resources (Clobert, Le Galliard, Cote, Meylan, & Massot, 2009; Kokko & Lopez-Sepulcre, 2006). Theory suggests that the evolution of dispersal, and the change in the dispersal strategies of a species, should reflect the spatial and temporal heterogeneity of the underlying habitat structure (Levin, Cohen, & Hastings, 1984; McPeek & Holt, 1992).

As distinct populations of a species often also face different habitat conditions across their geographic distribution, dispersal strategies might differ between populations of the same species; this would be indicated by a high intraspecific variation in dispersal (Stevens,

Pavoine, & Baguette, 2010). Likewise, various models predict the coexistence of more than one dispersal strategy according to habitat variability and heterogeneity (Bonte, Hovestadt, & Poethke, 2010; Doebeli & Ruxton, 1997; McPeek & Holt, 1992). If the fitness is equal between habitat patches, and the dispersal of resident individuals does not change this situation—populations are synchronized—there will be no selection for dispersal. In many situations, such as one where range margins are expanding (Thomas et al., 2001), populations experience nonequilibrium metapopulation dynamics. Here, a trait affects the ecological dynamics of a population, and the population dynamics in turn determines the selection pressure on this trait. In such cases, if the ecological conditions differed between habitat patches (Doebeli & Ruxton, 1997), the evolutionary branching of a population into distinct coexisting dispersal strategies is observed in a two-patch metapopulation model. Accordingly, dispersal strategies are strongly dependent on the quality of habitat patches. Dispersal should be selected only if individuals achieve fitness benefits through leaving the natal habitat and emigrating to another habitat patch. Theoretical models show that dispersal is not favoured under conditions that are temporally stable but spatially heterogeneous (e.g., Dieckmann, O'Hara, & Weisser, 1999; Kun & Scheuring, 2006; McPeek & Holt, 1992; Poethke & Hovestadt, 2002), because individuals might be more successful when they remain at the same site. In contrast, dispersal could be beneficial if the habitat conditions vary over time and are therefore uncertain (Bocedi, Heinonen, & Travis, 2012; Kun & Scheuring, 2006; Travis, 2001).

These theoretical considerations are also supported by empirical evidence. In natural populations of plant hopper species (*Prokelisia marginata* and *P. dolus*), wingless morphs were associated with stable habitats, whereas the presence of winged morphs was associated with habitat disturbance (Denno et al., 1996). Furthermore, in an experimental microcosm study, Friedenberg (2003) showed that an originally nondispersing strain of *Caenorhabditis elegans* evolved dispersal ability when confronted with fluctuating patch size and food supply. For the European diving beetles (Dytiscidae; genus *Bidessus*), which inhabit lentic and lotic aquatic habitat types, habitat-specific dispersal patterns shape their genetic population structure. In this system, the low dispersal ability of a species associated with a more stable lotic environment resulted in a higher number of genetic clusters and stronger geographic structuring when compared to a species found in lentic habitat types, which have evolved a higher dispersal tendency to cope with the unpredictable habitat conditions of ephemeral aquatic habitats (Papadopoulou et al., 2008).

Adaptation to new or changing habitat conditions might not only alter the morphology, behaviour and physiology of individuals, but also their dispersal tendencies. Intraspecific differentiation of dispersal behaviour has been shown in the context of habitat disturbance (Entling, Stämpfli, & Ovaskainen, 2011) and in the course of the invasion of a non-native species (e.g., Philips, Brown, Webb, & Shine, 2006). In theory, the partial improvement of habitat quality (in this case, habitat stability) would result in a decreased dispersal tendency of individuals, which would in turn reduce their ability to recolonize habitat patches (Poethke, Gros, & Hovestadt, 2011).

Environmentally dependent adaptation can cause the interruption of gene flow between individuals of differentially adapted ecotypes, and can ultimately lead to the formation of new species if reproductive isolation is finally achieved—a process known as ecological speciation (Dieckmann, Doebeli, Metz, & Tautz, 2004; Hendry, 2009). The stage at which dispersal behaviour changes during the course of ecological speciation has not yet been addressed. Here, we analyse the fine-scale genetic structure and parallel differentiation of dispersal (movement) behaviour in a large population of terrestrial fire salamanders (*Salamandra salamandra*) that is currently in a process of habitat-dependent adaptive divergence. A preliminary genetic analysis of salamander larvae suggested that this population has genetically differentiated into two subpopulations associated with the two larval habitat types inhabited (i.e., streams vs. ponds) (see Steinfartz, Weitere, & Tautz, 2007). By depositing their larvae in either permanent small, first-order streams or ephemeral water bodies (e.g., small temporary ponds), these salamanders show several habitat-specific adaptations to the different environmental conditions experienced by the larvae (Weitere, Tautz, Neumann, & Steinfartz, 2004). After metamorphosis, these salamanders are completely terrestrial—they live exclusively on land and can move freely through the entire forest.

We analysed how adaptation of the larvae to either environmentally stable streams or unpredictable pond habitats correlates with the overall movement behaviour of adult salamanders. We hypothesize that the movement strategies between stream- and pond-adapted salamanders have significantly altered as a result of adaptation to ecological differences between the preferred larval habitat types. To explore our hypothesis, we performed an individual-based fine-scale genetic analysis of larvae and adults across the whole forest. We integrate these results with the observed movement patterns as derived from radio-tracked individuals, and also by analysing the absolute distances travelled in the context of a capture–mark–recapture (CMR) study. Based on a demographic model, we further tested the impact of the genotypic habitat dependence on migration parameters. Our results indicate that movement behaviour can change and diversify at an early stage of adaptive divergence of a population into habitat-dependent, genetically differentiated subpopulations, and may be an important driver for the genetic divergence of populations.

2 | MATERIAL AND METHODS

2.1 | Study system

In Central Europe, the terrestrial fire salamander (*Salamandra salamandra*) is typically found in high abundance in old broadleaf forests, where they use small permanent primary streams as larval habitats. In the Kottenforst forest area, near Cologne and Bonn in West Germany (Figure 1), however, salamanders also use ephemeral habitats such as small ponds as larval habitat sites. Because the risk of desiccation is high and the food supply is comparably lower due to strong intraspecific competition—which may also result in a high frequency of cannibalism (Manenti, Pennati, & Ficetola, 2015; Reinhardt, Steinfartz, & Weitere, 2015)—larvae developing in the ephemeral habitats show several habitat-
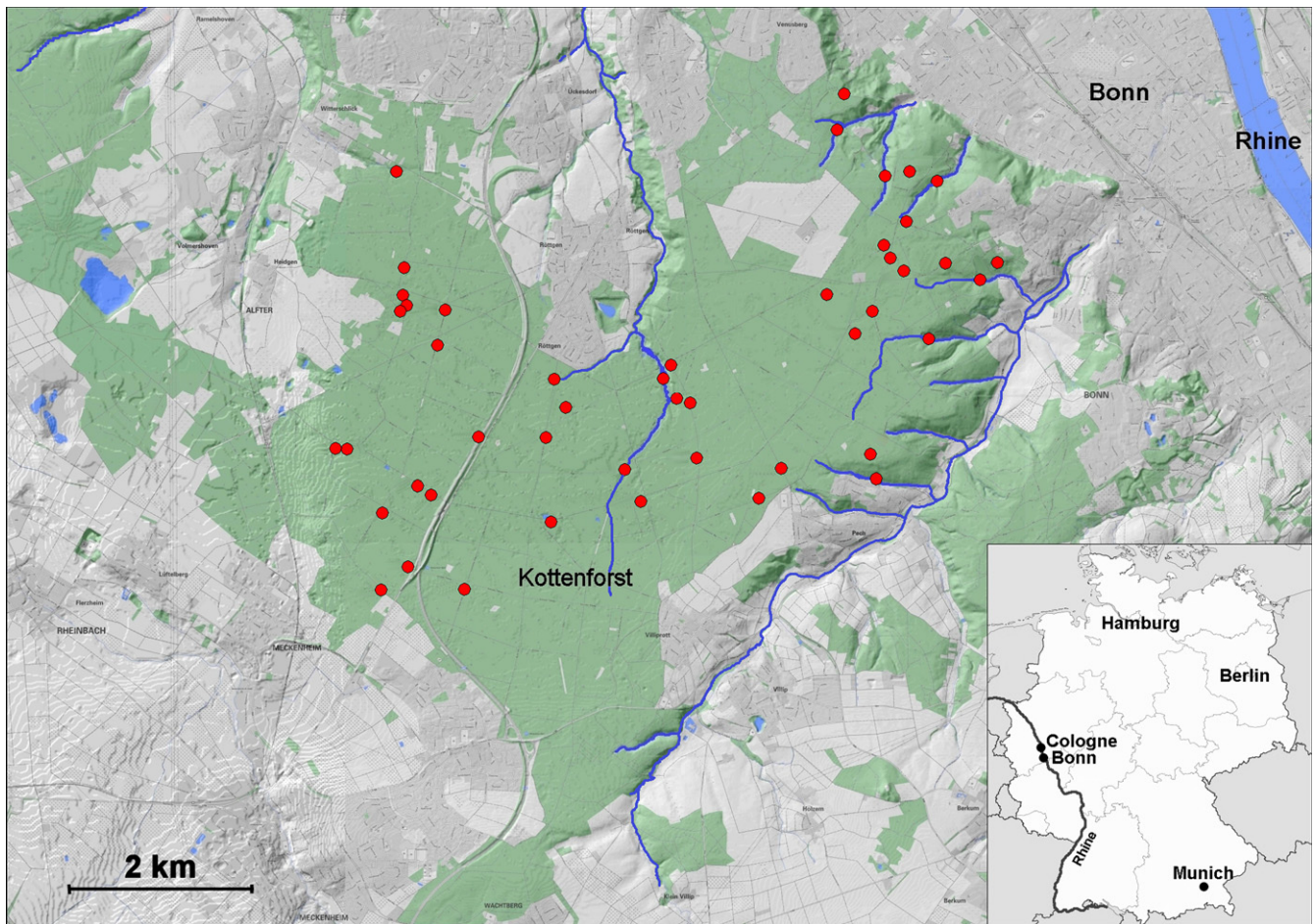
FIGURE 1 Map of the Kottenforst near Bonn in Germany. Area in green reflects old broadleaf forest. Dots indicate sampling sites inhabited by *Salamandra salamandra* larvae across the forest in ephemeral pond habitats (mainly in the west) or stream habitats mainly located at the eastern rims. A more detailed description of sampled sites is provided in Table S1

specific responses/adaptations that are absent in stream-adapted larvae. Under natural conditions, these responses/adaptations include a greater larval mass at birth, the ability to thrive on lower-quality food, and an early metamorphosis—which was also observed under common environmental conditions in the laboratory—all of which facilitate escape from unfavourable and unpredictable conditions (see Weitere et al., 2004 for details). In contrast, the conditions experienced by larvae in streams are more stable, because the food and water supply is consistently high (Thiesmeier & Grossenbacher, 2004).

In the Kottenforst forest, a continuous forest patch of approximately 30 km², the presence of two genetically differentiated subpopulations has been identified on the basis of 11 microsatellite loci. One subpopulation includes salamanders from stream habitats, whereas the other subpopulation comprises salamanders from ephemeral pools (Steinfartz et al., 2007). In detail, the Kottenforst forest lies on an uplifted plateau that is part of the old Pleistocene Rhine terrace and fades into rural environments to the west, hilly areas to the south and sharply declines to the Rhine River to the east. Most of the streams that originate within the Kottenforst forest are located in the marginal regions from the eastern to the southern slopes (see Figure 1). Ephemeral and perennial water bodies such as ditches, wallows from wild boars, tire ruts and pools can be found frequently across the Kottenforst forest, although they are more common in the western part of the forest. The Kottenforst forest does not present any spatial barriers to dispersal of adults and, accordingly, salamanders can move freely across the entire forest and are mainly active during rainy nights in the spring and autumn. The major highway that divides the western from the central part of the Kottenforst forest can be passed underneath by adults and drifting larvae, and no genetic differentiation linked to the course of the highway could be detected in a previous study (Steinfartz et al., 2007).

2.2 | Individual-based fine-scale genetic analysis of the Kottenforst population

In order to analyse the fine-scale genetic population structure of salamanders in the Kottenforst, we sampled larvae from 47 sites, representing ephemeral water bodies and streams during spring in 2007–2010 (a detailed overview is provided by Fig. S1 and Table S1). Larvae were genotyped for a set of 17 microsatellite loci

(Sal-E5, Sal-E6, Sal-E7, Sal-E8, Sal-E11, Sal-E12, Sal-E14, Sal3, Sal23, Sal29, II-A6, B11, C2, C3, E11, G6, G9) for which sequence information, details on PCR amplification and multiplexing of loci, as well as scoring of alleles, are described in detail in Steinfartz, Küsters, and Tautz (2004) and Hendrix, Hauswaldt, Veith, and Steinfartz (2010). The full data set of 2,563 individual larval genotypes were first analysed for failures of single allele amplification and genotyping errors using the software program MICRO-CHECKER version 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). For this data set, we used a Bayesian approach to estimate the number of genetic clusters (K) as implemented in the program STRUCTURE (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000). Parameters for the structure analysis were adjusted by using the admixture model; a separate alpha (α) was inferred for each population, and correlated allele frequencies estimated for each population, iterating $K = 1$–9 five times, without prior population information. Analyses were run for 800,000 generations, of which the first 100,000 were discarded as burn-in. We then used the online version of the program

STRUCTURE HARVESTER, web v0.6.94 (Earl & vonHoldt, 2012), to estimate the number of K populations according to Pritchard et al. (2000) and an ad hoc statistic ΔK, based on the rate of change in the log probability of data between successive K values according to Evanno, Regnaut, and Goudet (2005). We further analysed the genetic population structure by estimating F_{ST} differentiation and performing principal coordinates analysis by using the program GENALEX 6.503 (Peakall & Smouse, 2006, 2012). We estimated migration rates between subpopulations based on microsatellite loci data using the program BIMR (Faubet & Gaggiotti, 2008). BIMr makes inferences on gene flow among subpopulations and can estimate migration rates over the last generation based on a Bayesian approach using Markov chain Monte Carlo simulations. As such, migration rates were estimated between the three major geographic groupings inferred from the STRUCTURE analysis, representing the stream subpopulation (genetic cluster in the east of the Kottenforst), the pond subpopulation (genetic cluster in the west of the Kottenforst) and a contact zone between both subpopulations spanning an area centred around an
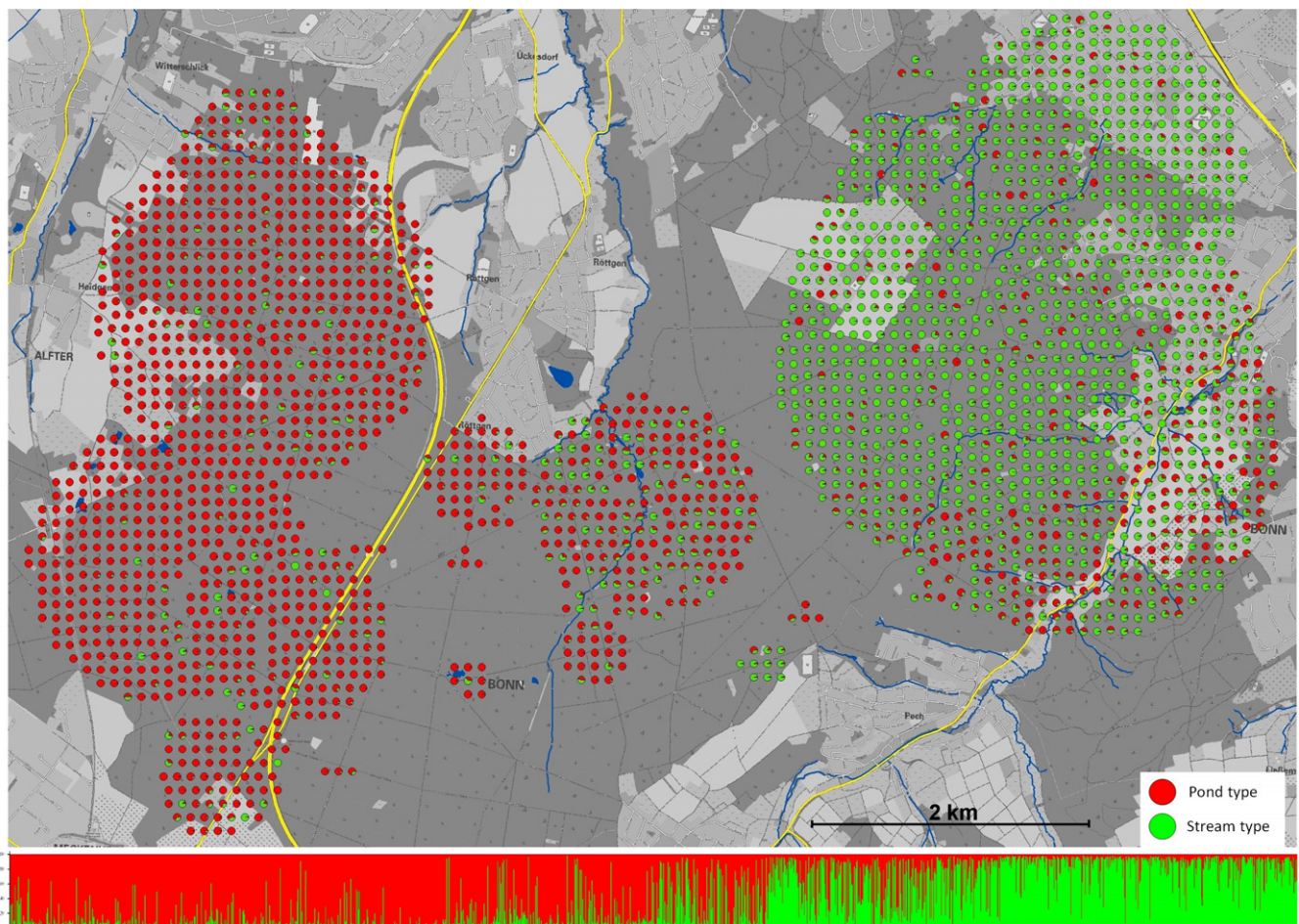

FIGURE 2 Fine-scale spatial distribution of 2,653 genotypes representing individual salamander larvae sampled from pond and stream habitats as shown in Figure 1. Each dot represents a single individual displaying as a pie chart the percentage assignment assuming two genetic clusters ($K = 2$) according to the program STRUCTURE. Positioning of dots were performed with an implemented option of the geographic information system program QGIS 2.18.4 for geographic coordinates of larvae sampled from a specific site. A detailed breakdown of individual larval genotypes according to specific sites is provided in Fig. S1 and Table S1. The barplot composed of individual genotypes (each line represents a single larvae) shows the corresponding assignment as represented by the pie charts from west to east across the Kottenforst

intermittent stream and including sampling sites indicated by signs of introgression (see Figure 2, Fig. S1 and Table S1). We calculated migration rates by using the default parameters of BIMr, except that we increased the number of replicates per run to 5.

In addition to the sampling of larvae, we also sampled adult salamanders during rainy nights of activity periods in spring (March to late May) and autumn (September to October) between 2007 and 2010 (for more details, see CMR methods below). A set of 1,106 adult individuals were genotyped and scored for the same set of 17 microsatellite loci as described above. Settings used in STRUCTURE were identical to those described for the larval analysis.

2.3 | Genetic habitat assignment of adults

As fire salamanders live a completely terrestrial life after metamorphosis, and cannot be associated with their original larval habitat, we assigned adults to their respective larval habitat type using a genetic assignment analysis. Accordingly, each adult individual was assigned to its respective larval habitat origin, that is, either as being pond-adapted or as stream-adapted, based on assignment to larval habitat clusters using microsatellite loci data. Of the larval data set sampled across the Kottenforst and genotyped for the 17 loci, reference groups representing both the stream-adapted subpopulation and the pond-adapted subpopulation were built. Each group was represented by 400 randomly chosen larvae that were assigned with at least 85% probability to either the stream subpopulation or the pond subpopulation as inferred by the program STRUCTURE. Genetic assignment of adults was performed using the program GENECLASS 2 (Piry et al., 2004), with its implemented Bayesian assignment approach, using a 50% threshold to assign each individual to either the stream- or the pond-reference group.

2.4 | Estimating movement patterns based on a CMR analysis of adult salamanders

One of the major aims of this study was to analyse the differentiation of movement patterns of adult salamanders in the context of adaptation to the larval habitat type and observed genetic population differentiation. We used a CMR approach to characterize the movement patterns of adult salamanders, and integrated this with our fine-scale genetic study by associating each individual with its respective larval habitat type (see below for details).

To obtain estimates of movement probabilities of salamanders, we randomly sampled subadult and adult salamanders along forest roads across the entire forest. Each individual was marked with a passive implantable transponder (PIT) tag to enable its unambiguous identification when recaptured. Four to five people with flashlights surveyed for salamanders during rainy or humid nights in spring/autumn of 2007 (seven nights), 2008 (19 nights), 2009 (14 nights) and 2010 (13 nights). Each captured individual was measured and if possible sexed. In addition, a tissue sample was taken from each for the genetic study, and the exact capture location was recorded using a GPS device (Garmin GPSMAP 64st) with an accuracy of within 5 m.

Salamanders with a minimum snout-vent length of 85 mm were fitted with a PIT-tag (Trovan ID100, 11.5 mm $\times$ 2.5 mm; Schulte, Küsters, & Steinfartz, 2007), marked and released at the original capture location. Coordinates of capture and recapture localities of the individuals are reported in the UTM grid, WGS84 format.

Based on capture–recapture data, we performed two major analyses. First, using a multistate capture–recapture model, we analysed the movement probabilities of the salamanders. Second, we analysed the observed moved distances. Our aim was to model the probability that salamanders moved a distance larger than d from one occasion to the next (Schaub, & van Hirschheydt, 2009). We therefore defined different states in the following way: all the individuals were in the "initial" state when they were captured for the first time; all the recaptures were classified as states "close" or "far," depending on whether the Euclidian distance between the location of the actual recapture and the location of the previous recapture (or capture) was lower or higher than d. We then formulated a multistate capture–recapture model (Lebreton, Nichols, Barker, Pradel, & Spendelow, 2009) that accounts for transient individuals (Pradel, Hines, Lebreton, & Nichols, 1997; Schaub, Liechti, & Jenni, 2004; Schmidt, Feldmann, & Schaub, 2005) and that allows for the estimation of the movement probabilities. The model contains three parameter types, namely the recapture probability (p), the apparent survival probability (ϕ) and the movement probability (ψ). Further details on the model and results on the goodness-of-fit assessment are provided in the Supporting Information. We used the program MARK 6.0 (White & Burnham, 1999) to analyse the data.

We varied the critical distance d (=25, 50, 75, 100, 125, 150, 200, 300, 400, 500, 600 m), as well as recoded and analysed the capture–recapture data. Using the data with $d = 25$ m, we initially performed a model selection in three steps: first, we modelled the recapture probability, then the survival and finally the movement. For each type of parameter, we fitted the models with interactive time and ecotype effects, additive time and ecotype effects, effect of time only, effect of ecotype only and constant. We kept the most parsimonious model structure, as identified by the quasi-likelihood Akaike information criterion (QAICc; Burnham & Anderson, 2002) from the previous parameter, to proceed with the model selection. For the data with distances $d > 25$ m, we did not model the recapture and survival, but we instead used the structure identified for the data with $d = 25$ m. This decision is justified because the recapture and survival are not state-specific and, therefore, are unlikely to have another structure regarding time and origin effects. In contrast, the movement is dependent on the states, and we therefore modelled movement for $d > 25$ m.

2.5 | Testing for short- and long-distance movement

Assuming salamanders that adapted to the unstable and less predictable pond habitat should disperse longer distances when compared with individuals adapted to the stable and predictable stream habitat, we tested, in a second analysis, whether the dispersal of

salamanders in the Kottenforst forest was habitat-specific, and can be classified as long- vs. short-distance movement. For each salamander that was recaptured at least once, we calculated the maximum travelled distance by adding the Euclidian distances between the chronological recapture events. The habitat association of recaptured individuals was performed using a genetic approach of assigning individuals into a pond-adapted group vs. a stream-adapted group as described above. For each group, the maximum travelled distances were plotted into frequency-dependent distance classes in categories of 100 m. We applied two regression-based models to test each group for a pattern of long- and short-distance dispersal. The pattern of short-distance movement was tested by a negative exponential function (NEF) that typically describes the shape of short-distance movements. In contrast, the long-distance movement should result in an L-shaped distribution of dispersal kernels, which is best approximated by an inverse power function (IPF according to Hill, Thomas, & Lewis, 1996; Baguette, 2003).

$$I_{NEF} = ae^{-bD} \text{ respective } \ln I = \ln a - bD$$
$$I_{IPF} = aD^{-b} \text{ respective } \ln I = \ln a - b(\ln D)$$

where I is the probability of an individual moving a certain or larger distance (D), a is the intercept and b is the slope of the regression of log-transformed cumulative proportions of individuals who moved certain distances, against the distances and log-transformed distances (see Kuras, Beness, Fric, & Konvicka, 2003). We used the F-statistics to determine whether the actual moved distances within each group of individuals fit better to either the NEF or the IPF, by applying a curve estimation function for the regression analyses in SPSS 20.0.

2.6 | Radio-telemetry

In addition to the CMR study, we analysed whether the home range size of adult individuals differs between ecotypes. Therefore, eight pond-adapted (three males and five females) and 12 stream-adapted (five males and seven females) individuals were fitted with implantable radio-transmitters of the BD-2H type (Holohil Systems Ltd., Carp, Canada) and PIP3imp-R (Biotrack Ltd., Dorset, UK), which had wrapped internal antennas. With a weight of 1.0–1.2 g, the radio-transmitters (ca. 16 mm × 8 mm × 5 mm) were, on average, 3.38% ($SD \pm 0.67\%$) of the body mass of the individuals. Each transmitter was implanted under the skin of the lateral-abdominal region by a veterinary surgeon and run with a reduced pulse rate of 30 pulses/min. Salamanders were kept in captivity for observation for 10 days following the surgery before they were released at their capture site. All the implanted individuals successfully recovered from the surgery and did not show any behavioural abnormalities in comparison with other captive salamanders. We aimed to point-track each individual, at least once per day using hand-held Yagi antennas (Televilt Y-4FL) in combination with bar antennas (Televilt GS antenna with 0.8 m range of signal detection) and tracking receivers (Televilt RX-98E; Followit Lindesberg AB, Lindesberg, Sweden). Five individuals were released and tracked in the spring of 2008, 10 in the spring of 2009 and five additional individuals in the autumn of 2009.

Of the 20 initially radio-tracked individuals, the home range size could be estimated for 17 individuals who were tracked over a period of 8–43 days. One individual was lost due to predation, and the transmitter batteries of two individuals were exhausted after 4–8 days. We used the RANGES7 software (South, Kenward, & Walls, 2008) with a 160 × 160 grid (cell size: 1 m²) and a tracking resolution of 8 m according to the accuracy of the GPS devices used. The fixed kernel (FK) method was used to estimate the core areas based on 50% and 95% kernel contours, according to the density distribution of located/tracked points. A least-squares cross-validation (LSCV) of the smoothing factor (h) was performed on the basis of four individuals, for whom at least 30 location records were available. In this way, the h value could be reliably estimated (Seaman et al., 1999). As the preliminary incremental area analyses indicated that at least 25 locations are required to obtain robust home range size estimates for the FK method, we applied the minimum convex polygon (MCP) method only to the 17 individuals who fulfilled these criteria. The MCP home ranges were calculated by drawing 95% convex polygons around the location points of each individual based on a recalculation of the arithmetic mean centre.

Differences in the estimated home range sizes according to the FK and MCP methods were statistically tested using a general linear model (GLM) in SPSS 22.0. We used the GLM to test for the effects of habitat and sex, as well as the interaction of both factors on home range differences. Full models were presented without the exclusion of nonsignificant effects (Forstmeier & Schielzeth, 2011). The MCP data were log-transformed (log ($x + 1$)) to obtain a normal distribution of the residuals. Normal distributions of residuals were tested using a Kolmogorov–Smirnov–Lilliefors test.

3 | RESULTS

3.1 | Fine-scale genetic structure and estimation of gene flow of the salamander population in the Kottenforst

Analysis of 2,563 larvae sampled from various pond and stream sites across the whole range of the Kottenforst population using STRUCTURE and STRUCTURE HARVESTER confirmed the presence of two genetically differentiated subpopulations (see Figure 2 and Fig. S2), as was previously suggested by Steinfartz et al. (2007). As shown by the spatial distribution of individual genotypes, larvae from pond sites in the western part demonstrate a high probability of belonging to a separate genetic cluster (i.e., subpopulation) than larvae sampled from streams in the eastern part of the Kottenforst, which represents a distinct genetic subpopulation. Intermixed individuals between these subpopulations are mainly found in the middle and in the southwest of the Kottenforst, which is characterized as "contact zone" (see Fig. S1 and Table S1 for details). In order to illustrate the degree of genetic differentiation, we have divided the spatial distribution of larvae into five subgroups (see Fig. S3). Observed differentiation of F_{ST} values ranged from 0.006 between larvae from streams in the southeast of the Kottenforst, up to 0.017 between larvae from

streams in the east and west of the Kottenforst (see Table S2). Furthermore, salamander larvae of the stream subpopulation had a higher proportion of private alleles (0.235–1.00) than larvae of the pond subpopulation (0.059–0.118) (see Fig. S3).

Principal coordinates analysis, based on the covariance of genetic distances, corroborated the genetic differentiation between subpopulations as inferred from the STRUCTURE analysis. Stream and pond subpopulations were separated by axis 1 and axis 2, explaining 8.84% of the observed genetic differentiation, while larval genotypes from the contact zone did not show separation at this level, thereby indicating an area of frequent hybridization between the two subpopulations (see Figure 3). Migration rates estimated with the program BIMR revealed a similar high value of 0.51 between sites within the area of the pond subpopulation, and between the pond subpopulation and the contact zone (0.47), whereas migration from the pond into the stream subpopulation was estimated to be only 0.011 (see Table 1). The highest value of estimated migration—indicative of high genetic homogeneity—was found within the stream subpopulation (0.70). Interestingly, migration from the stream into the pond subpopulation was estimated to be higher (0.20) than into the contact zone (0.10).

The fine-scale distribution of adult salamanders based on STRUCTURE analysis provided rather similar results as for the larvae; two subpopulations were inferred (see Figures S4 and S5). Individuals found in the western part of the Kottenforst also formed a distinct genetic cluster when compared to individuals found in the eastern part of the Kottenforst. In addition, the contact zone identified from the larval genotypes was confirmed by the adult genotypes.

3.2 | Genetic habitat assignment of adults used for modelling and analysis of movements

Genetic assignment of adults to reference groups representing the stream- or the pond-adapted subpopulation using a 50% cut-off resulted in overall high assignments. Accordingly, 75% of individuals were assigned to the stream- or the pond-reference group with at least 90% probability. Overall, 90% of the adults assigned to the pond-reference group had at least a 75% assignment probability and 90% of the adults assigned to the stream-reference group showed at least a 65% assignment probability (see Figure 4 for details).

3.3 | Analysis of monthly movement probabilities

Modelling the recapture and survival rates revealed that both parameter types were time-dependent, and the recapture probability included an additive ecotype effect (pond-adapted salamanders had slightly higher recapture probabilities; Table 2). For the modelling of movement probabilities, we found that the best models included an ecotype effect when the distances were less than 100 m (Table 3). When the critical distances to define dispersal states were 100 m or higher, the ecotype had no effect on the movement probabilities, which strongly declined with an increasing distance (d) and were higher in stream-adapted than in pond-adapted salamanders when short distances were considered (Figure 5).

3.4 | Habitat-dependent differentiation of movement patterns

The maximum distances travelled of 178 adult salamanders that were recaptured at least once ranged from below 10 m to 1.9 km, with a higher proportion of short movements than long-distance movement events (see Figure 6). However, if the individual movements of adults were analysed according to the associated habitat adaptation, that is, stream- or pond-type, we found that the movement patterns were shaped by habitat-dependent characteristics. Although the median distance moved by pond- and stream-adapted individuals was similar (around 200 m), 90% of pond-adapted individuals moved up to 700 m, and single individuals reached up to almost 2 km, while stream-adapted individuals did not move beyond 500 m from their first capture location (Figure 6a and Fig. S6). When specifically tested for long- vs. short-distance movement patterns, pond-adapted individuals better fitted the L-shaped distribution of the IPF ($R^2 = 0.96$, $F_{1,13} = 325.27$, $p < .01$)—which is indicative of long-distance movement—than the NEF ($R^2 = 0.66$, $F_{1,13} = 24.74$, $p < .01$), which typically characterizes short-distance movement (Figure 6b). In contrast, the movement of stream-adapted individuals
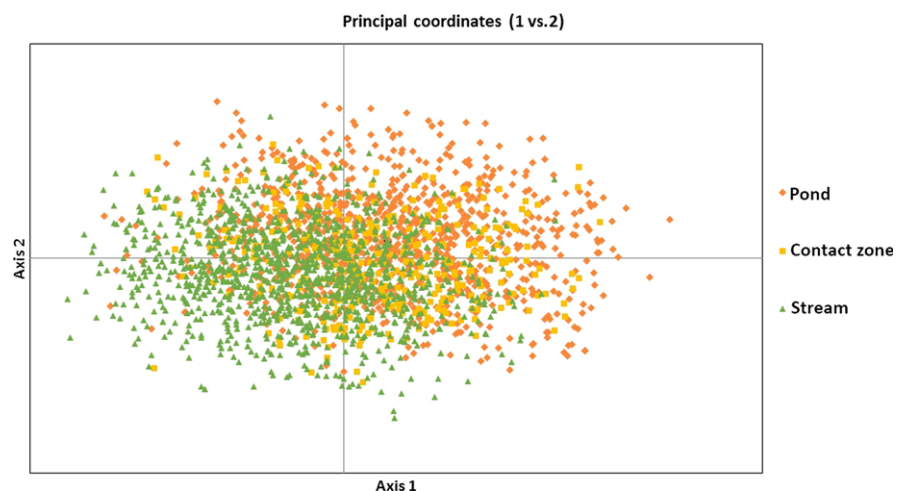
FIGURE 3 Principal coordinates analysis based on covariance of genetic distance inferred from microsatellite loci differentiation of larvae found across the Kottenforst. Shown are the first two axes, which are separating larvae of the pond and stream cluster, while larvae of the contact zone are not separated on that level (see Table S1 for definition of groups)

Principal coordinates (1 vs.2)

Axis 2 / Axis 1

- Pond
- Contact zone
- Stream

TABLE 1 Estimation of recent migration rates and gene flow between major subgroups of larval sites within the Kottenforst using the program BIMR. Subgroups represent larvae of the pond cluster ("pond"), from the "contact zone" and from the stream cluster ("stream") as shown in detail by Fig. S1 and Table S1

| Subgroups | Estimated migration rate | | | | | |
	run1	run2	run3	run4	run5	Mean
pond–pond	0.516	0.515	0.517	0.517	0.516	**0.51625**
pond–contact zone	0.473	0.474	0.472	0.472	0.473	**0.47275**
pond–stream	0.0107	0.0106	0.0107	0.0107	0.0108	**0.010675**
contact zone–pond	0.194	0.197	0.197	0.201	0.201	**0.19725**
contact zone–contact zone	0.502	0.501	0.501	0.497	0.494	**0.50025**
contact zone–stream	0.305	0.302	0.302	0.302	0.305	**0.30275**
stream–pond	0.201	0.201	0.203	0.206	0.206	**0.20275**
stream–contact zone	0.103	0.103	0.101	0.0988	0.0985	**0.10145**
stream–stream	0.696	0.696	0.696	0.695	0.696	**0.69575**
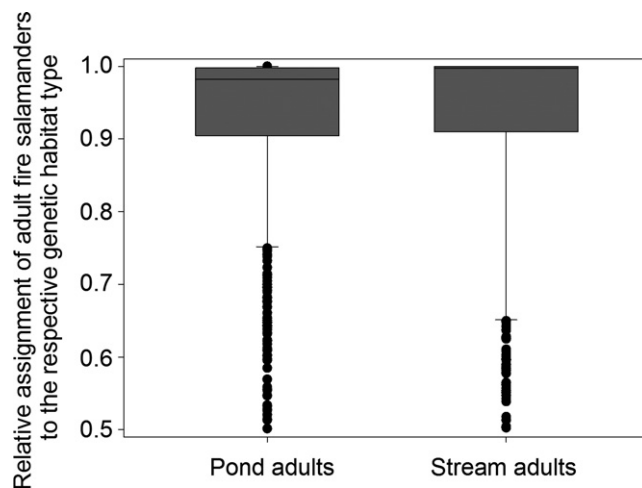
FIGURE 4 Box–whisker plot diagram showing the relative assignment of adults to reference groups of stream and pond larvae. Boxes indicate the 75% percentile and the 25% percentile, and the line in the box represents the median (i.e., 50% percentile). The lower whiskers indicate the range of 10% and the upper whisker 90% of the data, and dots indicate outliers of these ranges

was better described by the NEF ($R^2 = 0.95$, $F_{1,3} = 52.19$, $p < .01$) than by the IPF ($R^2 = 0.78$, $F_{1,3} = 14.77$, $p = .055$; Figure 6c).

3.5 | Home range estimates based on telemetry data

The estimates of home range size for the 17 radio-tracked individuals ranged between 106 and 26,788 m² (mean = 6,288 ± 7,743 SD) for the 95% fixed kernel method, and between 429 and 14,594 m² (mean = 3,894 ± 3,755 SD) for the MCP method (Figure 7 a,b). We found that the home range sizes differed between ecotypes. Pond-adapted individuals occupied larger home ranges (based on the FK method; GLM$_{FK}$: factor habitat type: $F_{1,13} = 5.47$, $p = .036$; factor sex: $F_{1,13} = 0.52$, $p = .48$; interaction habitat type*sex: $F_{1,13} = 1.79$, $p = .20$; R^2 of the model = 0.34; Figure 7a). For the MCP method, the home range size did not significantly differ between stream- and

TABLE 2 Model selection results for recapture (p) and survival (ϕ', ϕ) using data with $d = 25$ m. The structure for the movement probabilities was in all models Ψ_{oxt}, where e denotes ecotype and t time. Given are the model deviance, the number of estimated parameters (K), the ΔQAICc and the Akaike weights (w). Note that $\hat{c} = 1.45$

Model	QDeviance	K	ΔQAICc	w
ϕ'_t, ϕ_t, p_{e+t}	1,110.64	77	0.00	0.60
$\phi'_{e+t}, \phi_{e+t}, p_{e+t}$	1,109.32	78	0.78	0.40
$\phi'_{ext}, \phi_{ext}, p_{e+t}$	1,067.71	105	16.04	0.00
$\phi'_{ext}, \phi_{ext}, p_t$	1,084.16	104	30.36	0.00
$\phi'_{ext}, \phi_{ext}, p_{ext}$	1,049.43	122	34.02	0.00
$\phi'_., \phi_., p_{e+t}$	1,278.95	41	93.87	0.00
ϕ'_e, ϕ_e, p_{e+t}	1,277.40	43	96.41	0.00
$\phi'_{ext}, \phi_{ext}, p_e$	1,268.07	98	201.57	0.00
$\phi'_{ext}, \phi_{ext}, p$	1,284.29	97	215.67	0.00

pond-adapted individuals, but the effect was in the same direction as with the FK method; that is, pond-adapted salamanders occupied larger home ranges (based on MCP method, GLM$_{MCP}$: factor habitat type: $F_{1,13} = 1.52$, $p = .24$; factor sex: $F_{1,13} = 0.41$, $p = .53$; factor habitat type*sex: $F_{1,13} = 0.50$, $p = .49$; R^2 of the model = 0.20; Figure 7b).

4 | DISCUSSION

The adaptation of individuals to different habitat types is known to affect several traits that optimize survival and reproduction. Adaptation to different habitats can lead to an initial genetic divergence between and within populations, which in certain cases, could result in reproductive isolation and speciation (Hendry, 2009; Schluter, 2001). In the context of habitat-dependent divergence processes, the change in morphological and behavioural traits has been the primary focus of research. Studies in different natural animal systems show that morphological adaptations of the feeding apparatus enable individuals to optimize foraging in their respective habitats. The

TABLE 3 Model selection results for the movement probability (Ψ) of salamanders in relation to ecotype e and time t for different distance classes. The structure of recapture and survival was in all evaluated models ϕ'_t, ϕ_t, p_{e+t} as identified in Table 1. Given are the Akaike weights and the number of model parameters (K). The best models are highlighted in boldface for each distance class

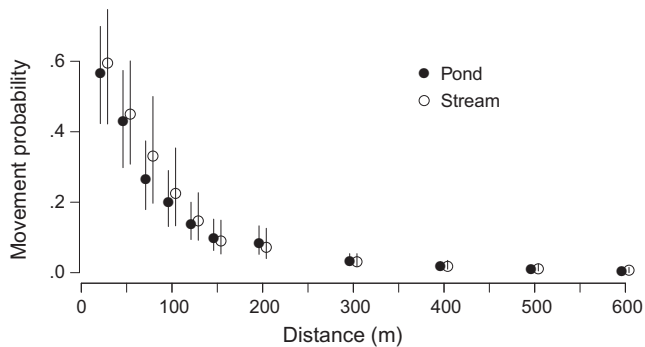|		Distance										
Model	K	25	50	75	100	125	150	200	300	400	500	600
Ψ_e	51	**0.97**	**0.62**	**0.55**	0.00	0.00	0.00	0.02	0.19	0.27	0.25	0.27
Ψ_{e+t}	64	0.02	0.01	0.05	0.49	0.38	0.27	0.24	0.07	0.00	0.01	0.00
$\Psi.$	50	0.01	0.37	0.37	0.00	0.00	0.00	0.05	**0.53**	**0.73**	**0.70**	**0.71**
Ψ_t	63	0.00	0.00	0.02	**0.51**	**0.62**	**0.73**	**0.69**	0.20	0.00	0.04	0.02
$\Psi_{e \times t}$	77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIGURE 5 Monthly movement probabilities for different distance classes of pond- and stream-adapted salamanders. Given are the mean and the limits of the 95% confidence intervals. All estimates are from models with structure

adaptation of beak morphology during the evolution of Darwin's finches on the Galápagos islands (Grant & Grant, 2008), or the diversification of the jaw and tooth morphologies during the adaptive radiation of cichlid fishes in the East African lakes (reviewed by Kocher, 2004), are both outstanding examples of this type of habitat-dependent adaptation, which can also occur on fine temporal scales (e.g., Barluenga, Stölting, Salzburger, Muschick, & Meyer, 2006). At the behavioural level, habitat-dependent mate choice is recognized as an important mechanism to achieve prezygotic isolation of individuals over the course of adaptive divergence (Coyne & Orr, 2004). However, habitat adaptation might not only affect the morphology and behaviour of diversifying groups of individuals, but also other important life history traits. Although theoretical and empirical studies have shown that dispersal of a wide range of organisms can be strongly influenced by habitat structure and availability, no study thus far has analysed whether and to what degree dispersal and movement are influenced in the context of habitat-dependent genetic differentiation and speciation. Here, we analysed how movement patterns and home range size of individuals differed in the context of habitat adaptation that is promoting genetic differentiation in a salamander population.

4.1 | Habitat adaptation promotes genetic differentiation in a salamander population

Fine-scale genetic analysis of more than 2,500 salamander larvae sampled across the whole Kottenforst confirmed the presence of

two genetically differentiated subpopulations with a $K = 2$ as found best option by STRUCTURE HARVESTER (see Fig. S2). Larvae sampled from ephemeral pond-like habitats in the west of the Kottenforst form a genetically distinct subpopulation from larvae found in permanently running streams in the east of the Kottenforst. We could further identify a contact zone of both subpopulations in the middle of the Kottenforst (Figures 2 and S1). Interestingly, this contact zone is centred around a periodic stream, which can be characterized as an intermediate habitat type between the pond and stream habitat, as the water flow is interrupted into isolated stagnant puddles during the course of the spring. Moreover, we found evidence of introgression of pond genotypes into the most southerly located streams in the east of the Kottenforst (see Figure 2).

Principal coordinates analysis—based on covariance of genetic distance—offered a more detailed insight into possible connectivity and gene flow between subpopulations. Although the separation between stream and pond subpopulations was not strong, explaining just under 9% of observed genetic variation, we see a clear pattern of genetic divergence along both axes (see Figure 3). Conversely, individuals sampled from the contact zone did not show a separation on that level, suggesting that gene flow between both subpopulations occurs mainly through the contact zone. Analysis of recent migration events with the program BIMR gave similar results. Estimated migration rate was highest within subpopulations, with 0.51 for the pond and 0.70 for the stream subpopulation, whereas migration rates between both subpopulations were low (0.01 for pond to stream and 0.20 for stream to pond; see Table 1). Possible gene flow from the pond into the stream population might occur via migration into the contact zone (0.47), and from there into the stream subpopulation (0.30).

Although observed genetic differentiation matches well with the type of larval habitat (i.e., pond-like vs. stream), the geographic occurrence of ponds in the west and streams in the east of the Kottenforst also matches with a pure geographic pattern—which could be an additional underlying reason for the observed genetic differentiation. Previous studies have documented an unexpectedly high dispersal ability of fire salamanders (Schmidt, Schaub, & Steinfartz, 2007; Schulte et al., 2007), and the movement of up to 1.9 km by adult salamanders in the course of our study (Figure 6) clearly questions the influence of geography as the basis for observed genetic differentiation in a system like the Kottenforst, where salamanders can move around freely and should interbreed with each other if
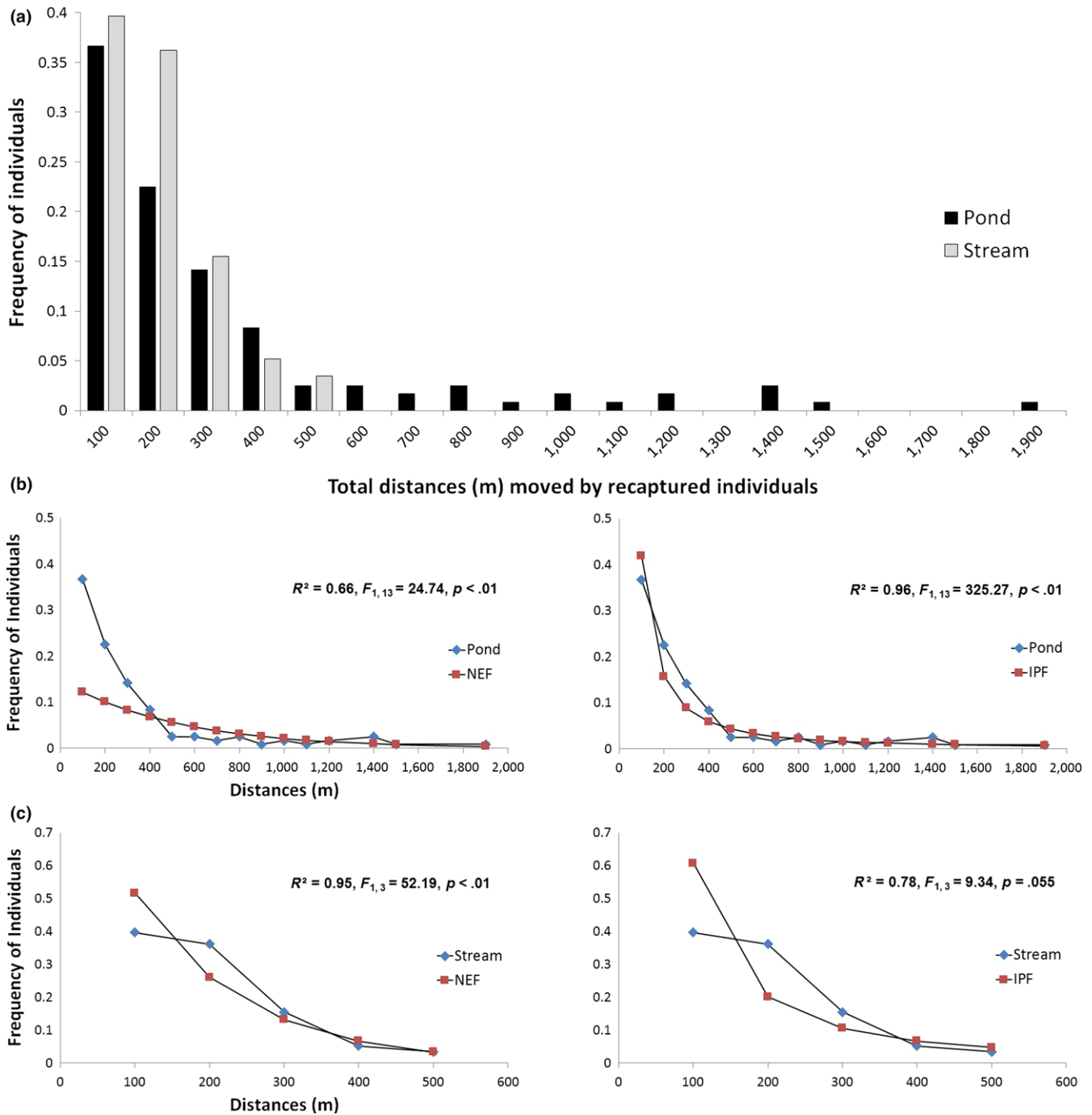
FIGURE 6 Analysis of movement patterns based on capture histories of 178 individuals. (a) Absolute moved distance across the Kottenforst. Long-distance movement has only been observed in pond-adapted individuals. (b) Observed movement pattern of pond-adapted individuals is better correlated and supported by the IPF (indicative for long-distance movements) than by the NEF (indicative for short-distance movements). (c) Stream-adapted individuals show a converse correlation and support

mating is random. Moreover, our fine-scale genetic analysis of adults showed that individuals of both subpopulations could be found in close geographic proximity to each other (e.g., in the east of the Kottenforst; Fig. S4). These two findings alone make random mating in the light of the observed genetic differentiation rather unlikely. We therefore have to assume that mating is nonrandom and most likely connected with larval habitat adaptation. In a recent study—analysing patterns of paternity and fitness of female salamanders in the

Kottenforst—polyandrous females showed mating preferences for males that were genetically more similar to each other (Caspers et al., 2014). As a background for the discussion of differences in dispersal and movement behaviour found by our study, we conclude that observed genetic differentiation is driven and maintained by the adaptation of larvae to the drastically different environmental conditions of the two habitat types.
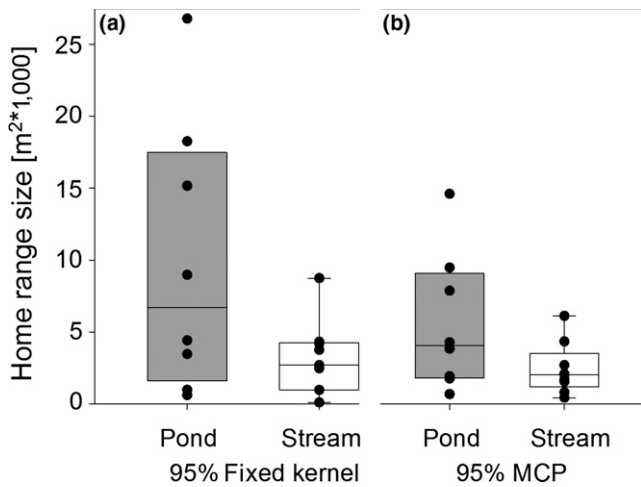
FIGURE 7 Averages of estimated home range sizes of 17 fire salamanders of two different types of habitat adaptation (i.e., pond [$n = 8$]- or stream [$n = 9$]-adapted). Home range estimates are based on radio-tracking data and are calculated and shown for the (a) fixed kernel method (factor habitat type $p < .05$) and (b) the MCP method (factor habitat type $p > .1$). Dots show individual data points, whereas the boxes indicate the 75% percentile and the 25% percentile, and the line in the box represents the median (i.e., 50% percentile)

4.2 | The differentiation of movement patterns in the light of habitat adaptation

Fire salamanders in the Kottenforst provide an exciting system to study the process and consequences of habitat adaptation with respect to changes and differences in ecology and evolution on the level of diversifying subpopulations, such as in the context of adaptive gut microbial diversity (Bletz et al., 2016) and gene expression differences between pond and stream habitats (Goedbloed et al., 2017). In a recent study, we showed that larval deposition behaviour and maternal investment differ between pond- and stream-type females. Pond-adapted females extended larval deposition over an increased period of time, with more frequent deposition events than stream-adapted females. Moreover, the body condition of larvae differed through deposition events and the body condition of stream-adapted females decreased more rapidly than pond-adapted ones (Caspers, Steinfartz, & Krause, 2015). We interpreted these observed differences as part of a bet-hedging strategy of females, developed to cope with the spatial (i.e., whether a pond-like habitat is available at all) and temporal (i.e., when a pond-like habitat is available) uncertainties of pond habitats, as compared to the more predictable conditions of stream habitats.

In principle, one could expect that movement and dispersal should also be influenced by the same kind of environmental uncertainty of pond-like habitats in contrast to stream habitats. We therefore modelled movement rates and analysed movement shapes and home range size of adults, with respect to larval habitat adaptation. By performing a genetic assignment of 1,106 adults to larval reference groups representing the pond and stream subpopulation, we determined their belonging to one of the subpopulations. We

decided to use a 50% assignment threshold in order not to exclude any individual, especially in the light of the more rarely recaptured adults. Given the high genetic assignment values (75% were assigned with more than 90% probability either to the stream- or to pond-reference cluster), and a rather similar distribution (see Figure 4)—which should account for potentially wrongly assigned individuals with a low assignment values in both directions—we consider 50% to be a reasonable threshold.

Habitat adaptation had no effect on overall movement probability; though, the probability to move short distances <100 m was higher for stream-adapted salamanders (Figure 5). This result is also confirmed by the findings regarding absolute distances moved. Here, stream-adapted individuals display a pattern of short-distance dispersal, while pond-adapted adults moved long distances up to 1.9 km with a typical movement pattern shape indicative of long-distance dispersal (see Figure 6). Interestingly, estimated migration rates based on genetic data also indicated higher values between stream sites (0.70) compared to sites of the pond subpopulation (0.51). Indeed, stream sites are geographically more closely located to one another, and the observed higher dispersal activity of stream-adapted salamanders for short distances might well explain the high migration rate inferred from the genetic data.

Although movement probability per se did not significantly differ between ecotypes, the variance in the travelled distances was higher in pond-adapted individuals (Figure 5), which indicates a higher flexibility of dispersal behaviour. Analysis of home range size of 17 adult radio-tracked individuals provided similar results; here, the variance in home range size was greater in pond-adapted individuals (see Figure 7). This increase in variance is interesting for two major reasons. First, as stream reproduction is widespread for *S. salamandra* populations in Central Europe, and pond reproduction has only evolved locally following the last glaciation in specific populations (approximately 8,000 years ago; Weitere et al., 2004), it can be assumed that stream reproduction is the ancestral state of the original salamander population in the Kottenforst and that variance in movement rates and distances must have increased with adaptation to pond reproduction. Second, in the very beginning of pond adaptation, that is, when stream-adapted females first started to deposit in pond-like habitats—perhaps as part of a bet-hedging strategy—successfully metamorphosed larvae from ponds might have returned or even have searched for this habitat type as adults, possibly due to habitat imprinting during the larval phase. In this early stage of habitat adaptation and later, we can consider plasticity—with its unique ability to generate an immediate phenotype on the basis of the same genotype—as an ideal response to the new environment and its requirements (West-Eberhard, 2003). For fire salamanders, it was shown that plasticity of foraging modes was particularly high if larvae from streams colonized a new habitat type (e.g., a cave environment), with drastically different environmental conditions (Manenti, Denoel, & Ficetola, 2013). If genetic variation in plasticity exists (or arises), and selection can favour certain phenotypes, then plasticity can promote population divergence and even speciation (Pfennig et al., 2010). The increased variance in movement and dispersal seen for pond-

adapted individuals might facilitate the rise of genetic variation, and thus promote the divergence of differentially adapted subpopulations. With the current data, it is difficult to say to what degree dispersal as a trait is phenotypically plastic or genetically controlled. To test for this, movement experiments under common environmental conditions—as performed for the larval deposition behaviour of females (see Caspers et al., 2015) or larval development (see Steinfartz et al., 2007 and Weitere et al., 2004)—should be carried out; however, such experiments are rather difficult to realize.

Given the observed genetic differentiation in the context of differential larval habitat adaptation in the Kottenforst, we can be quite sure that important traits to optimize fitness are under selection, and are at least partially genetically controlled, as may be the case for metamorphic timing and larval deposition behaviour. Therefore, it is reasonable to assume that movement and dispersal of salamanders in the Kottenforst are also under selection.

5 | CONCLUSION

We conclude that the differentiation of movement and dispersal behaviour as observed in a salamander population is the consequence of the divergence of this population due to larval habitat adaptation into subpopulations. The increased rate of variation measured for different traits of dispersal and movement of adults could be an important basis for the selection of phenotypically plastic traits. This process could be crucial for the evolutionary diversification of traits associated with the adaptation to new and changing environmental conditions met in many natural populations.

DATA ACCESSIBILITY

Microsatellite genotypes along with geographic coordinates of sampling sites, files used for the analysis with MARK and dispersal and home range data: Dryad https://doi.org/10.5061/dryad.h0r6q.

AUTHOR CONTRIBUTIONS

S.S., M.S. and B.R.S. designed the study. R.H. collected data in the field. R.H., M.S. and B.R.S. modelled monthly movement probabilities. R.H. analysed movement behaviour and performed genetic analyses. E.T.K. analysed home range size data. S.S., B.R.S., M.S. and R.H. wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Supplemental Information for:

Differentiation of movement behavior in an adaptively diverging salamander population

Ralf Hendrix, Benedikt R. Schmidt, Michael Schaub, E. Tobias Krause & Sebastian Steinfartz

Description of the multistate capture-recapture model

Model description

We estimated the probability that salamanders moved at least a certain distance within the study area with a multistate capture-recapture model (Lebreton et al. 2009) that accounts for the presence of transients (Pradel et al. 1997, Schaub et al. 2004). Transients are individuals that are present in the study area only at initial capture, thus have emigrated permanently from the study area after first capture. At first capture all individuals are assigned to state "initial", transient individuals leave the study area thereafter while non-transient individuals stay, but may move within the study area. To model movement, the model also includes two geographical states, which are defined based on the location of the individual with respect to the location of the previous encounter: if the Euclidian distance between the actual location of individual *i* and that of the previous location is less than the predefined distance *d*, it is coded as being in state "close", while it is coded in state "far" if the difference is larger than *d*. The transition to the state "far" then indicates that the individual has moved, while a transition to the state "close" indicates that the individual has not moved. A similar model has been used to study breeding dispersal of swallows (Schaub and von Hirschheydt 2009). The state transition matrix for the first time step after initial capture is

$$\begin{bmatrix} 1-\phi' & \phi'(1-\psi) & \phi'\psi \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix},$$

where the states of departure are in rows and the states of arrival in columns and the states are "initial", "close", and "far" from top to down and from left to right. The transition matrices thereafter are then all

$$\begin{bmatrix} 1 & 0 & 0 \\ 0 & \phi(1-\psi) & \phi\psi \\ 0 & \phi(1-\psi) & \phi\psi \end{bmatrix},$$

where ϕ is the apparent survival probability of non-transients, ψ is the movement probability of non-transients, and ϕ^{I} is a mixture parameter composed of the apparent survival probability of the non-transients and the proportion of transients τ, i.e. $\phi^{I} = \phi(1-\tau)$ (Pradel et al. 1997). The model for the observations is

$$\begin{bmatrix} 0 & 0 & 1 \\ p & 0 & 1-p \\ 0 & p & 1-p \end{bmatrix},$$

the states are in rows and the observations ("recaptured closed", "recaptured far", "not recaptured") are in columns and where p is the recapture probability. Note that the recapture probability is assumed to be independent of distance. This can be justified by the fact that the effort was spatially more or less homogenous and that whether or not a recapture was recoded as "close" or "far" depended on the location of the last recapture of that individual. The target parameters can be time-dependent (different at each occasion) and depend on the ecotype of the salamanders. We adjusted for the unequal time intervals between capture occasions to get estimates of monthly probabilities.

Goodness of fit

We assessed the goodness-of-fit of the general multistate model with interacting time and ecotype effects on all parameters with program U-CARE (Pradel et al. 2003, Choquet et al. 2009). The test indicated slight lack of fit (χ^{2}_{66} = 95.38, p = 0.009). The resulting overdispersion coefficient $\hat{c}$ = 1.45 was used for the model selection and the adjustments of the standard errors.

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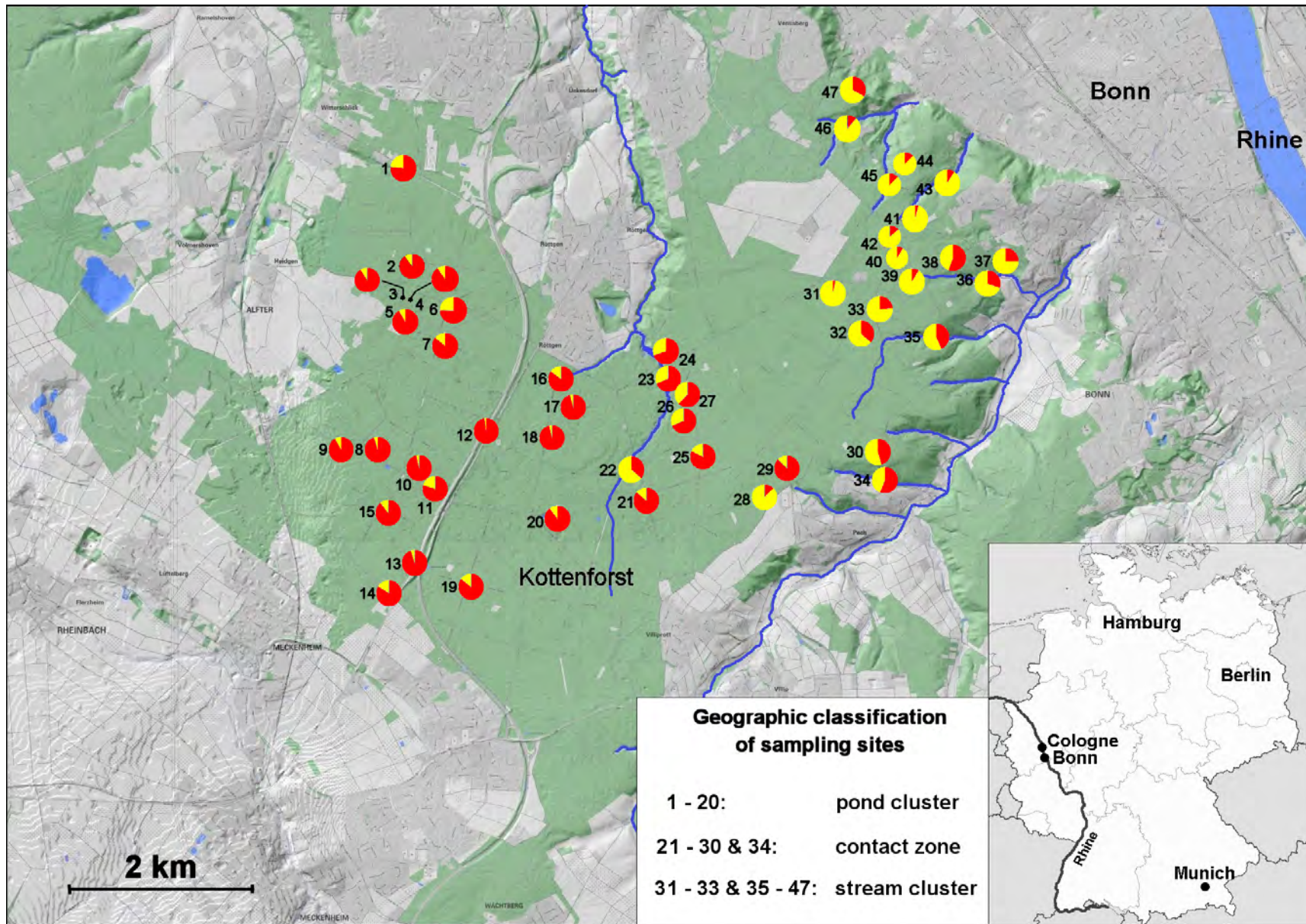
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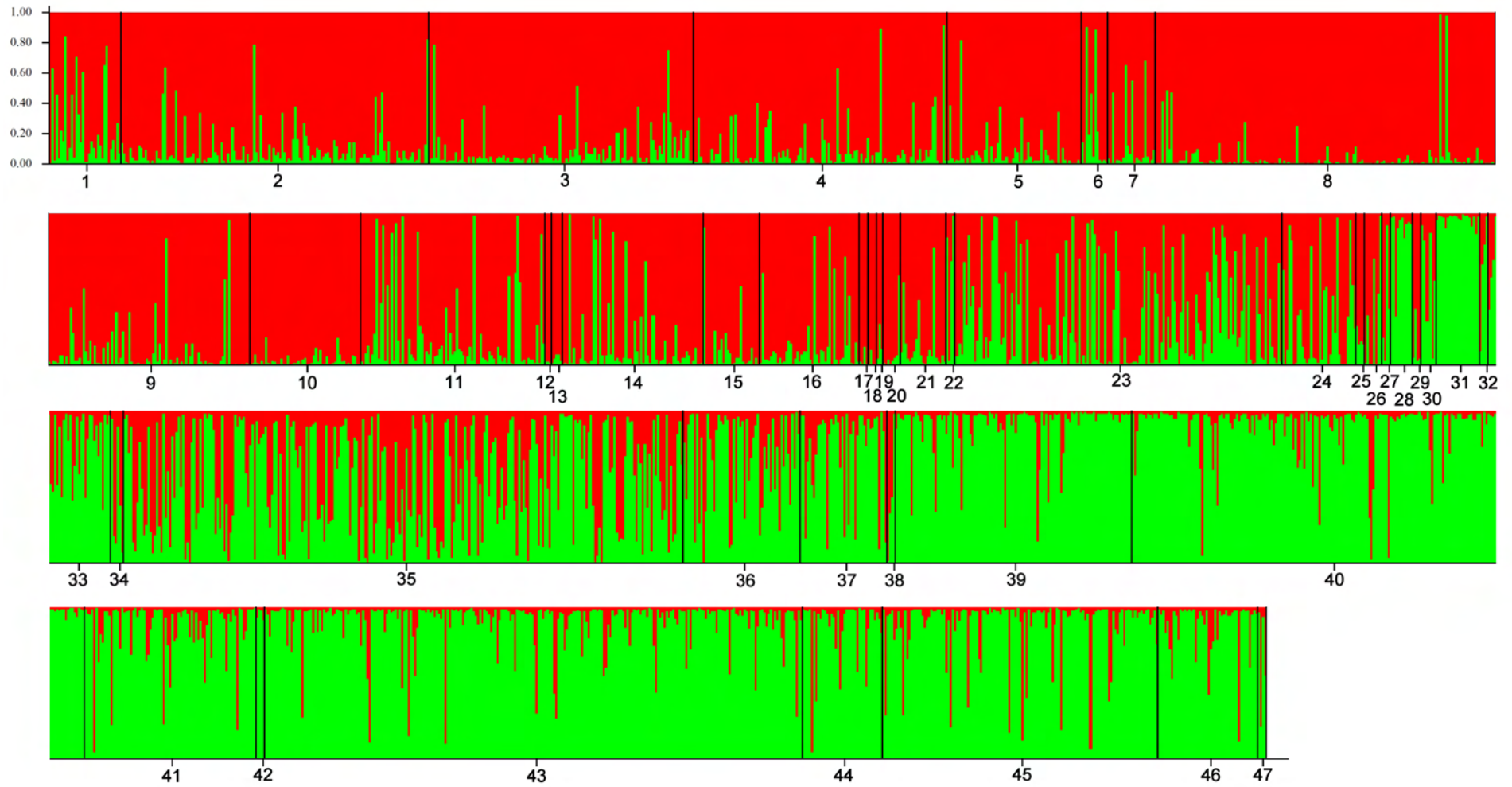
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Bonn

Rhine

47
46
45   44
43
41
42   38   37
40   39   36
31   33
32   35

1

2
3   4   6
5
7

16
17
18

24
23
26   27

25

29   30
28   34

12

9   8
10
11
15

22
21

20

13
14   19

Kottenforst

2 km

Geographic classification
of sampling sites

1 - 20:   pond cluster

21 - 30 & 34:   contact zone

31 - 33 & 35 - 47: stream cluster

Hamburg

Berlin

Cologne
Bonn

Rhine

Munich

Supplementary Figure 1: Sampling sites of salamander larvae across the Kottenforst with belonging to their respective main clusters (above). Corresponding assignment of individuals for each sampled location by STRUCTURE for K=2. Each line represents a single individual (see Supplementary Table 1 below for further details).

Cluster	Sampling site	No. of sampled individuals	Larval habitat	Total no. of sampled indv. per habitat cluster	No. of indv. assigned to pond or stream by Structure (probabilty >=50%)		pond (total) per cluster	stream (total) per cluster
					pond	stream		
Pond Cluster	1	33	ditch		27	6		
	2	142	ditch		139	3		
	3	122	ditch		119	3		
	4	117	ditch		114	3		
	5	62	ditch		60	2		
	6	12	pond		10	2		
	7	22	ditch		19	3		
	8	157	pond		155	2		
	9	93	pond		89	4		
	10	51	pond		51	0		
	11	85	pond	1060	71	14	1003	57
	12	3	pond		3	0		
	13	5	pond		5	0		
	14	65	ditch		58	7		
	15	26	pond		24	2		
	16	46	ditch		41	5		
	17	4	pond		4	0		
	18	4	ditch		4	0		
	19	3	pond		3	0		
	20	8	pond		7	1		
Contact Zone	21	21	pond		19	2		
	22	4	ditch		1	3		
	23	151	intermittent stream		108	43		
	24	34	pond		25	9		
	25	4	pond		4	0		
	26	8	pond	253	6	2	178	75
	27	4	tire rut		3	1		
	28	10	puddle		1	9		
	29	4	ditch		4	0		
	30	7	pond		3	4		
	34	6	stream		4	2		
Stream Cluster	31	20	pond		0	20		
	32	4	pond		1	3		
	33	32	ditch		6	26		
	35	258	stream		116	142		
	36	54	stream		16	38		
	37	40	pond		8	32		
	38	4	tire rut		3	1		
	39	109	pond	1250	7	102	200	1050
	40	184	tire rut		8	176		
	41	79	pond		6	73		
	42	4	ditch		0	4		
	43	248	stream		11	237		
	44	37	pond		4	33		
	45	127	stream		11	116		
	46	46	stream		2	44		
	47	4	puddle		1	3		

Supplementary Table 1: Designation of sampling sites to main genetic clusters and groups, number of sampled individuals per site, habitat type and assignment of individuals based on STRUCTURE for K=2 (pond *versus* stream cluster).

Supplementary Figure 2: Results of STRUCTURE HARVESTER for 2563 larval genotypes.

L(K) (mean +- SD)

DeltaK = mean(|L''(K)|) / sd(L(K))
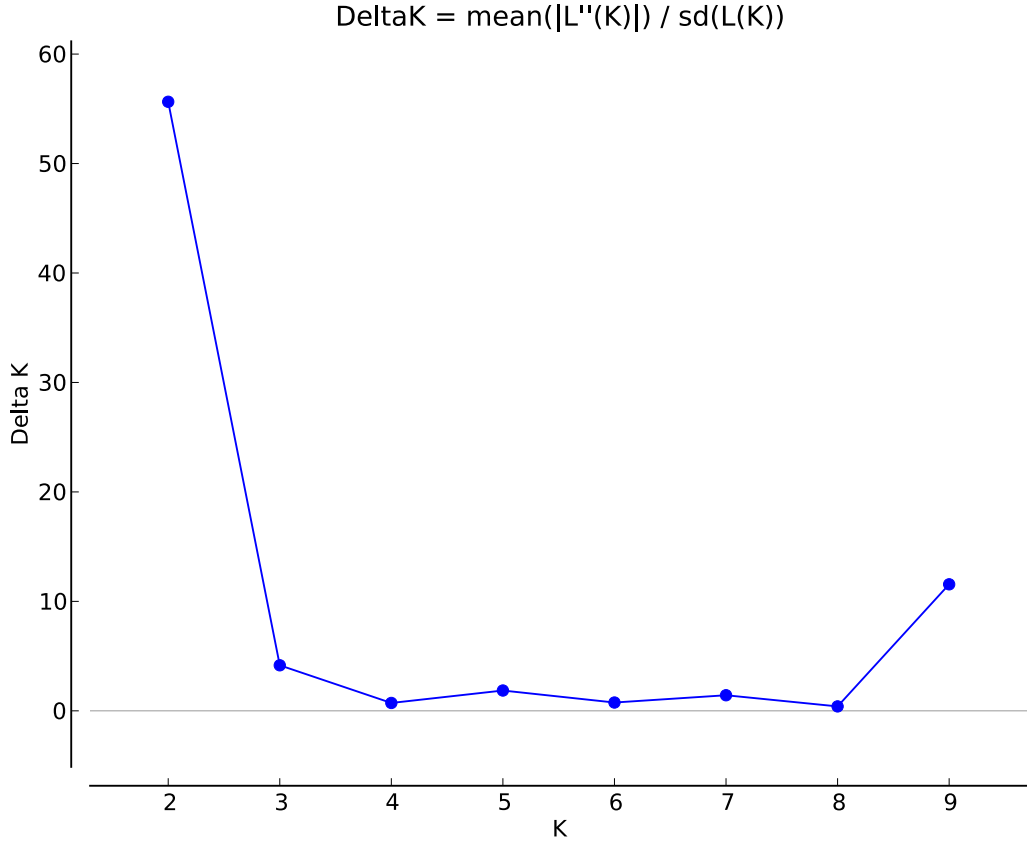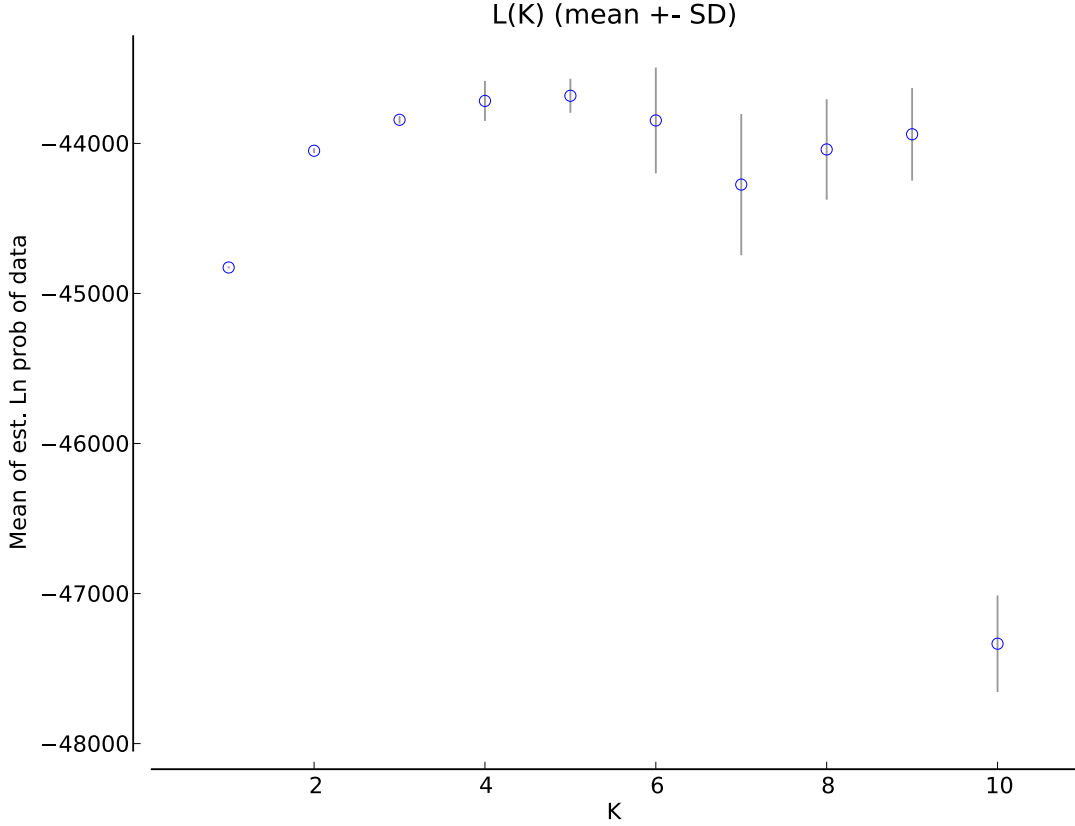
Supplementary Figure 3: Allelic patterns of pond (1, 2) intermediate (3) and stream (4, 5) genotypes of salamander larvae based on results of STRUCTURE (K=2) from different geographic locations across the Kottenforst calculated with the program GENALEX version 6.5. **Na** = Number of different alleles; **Na (Freq ≥ 5%)** = Number of different Alleles with a frequency ≥ 5%; **Ne** = Number of effective Alleles = 1 / (Sum pi^2); **No. Private Alleles** = Number of alleles unique to a single population; **No. LComm Alleles (<=50%)** = Number of locally common alleles (Freq. ≥ 5%) found in 50% or fewer populations; **He** =Heterozygosity.
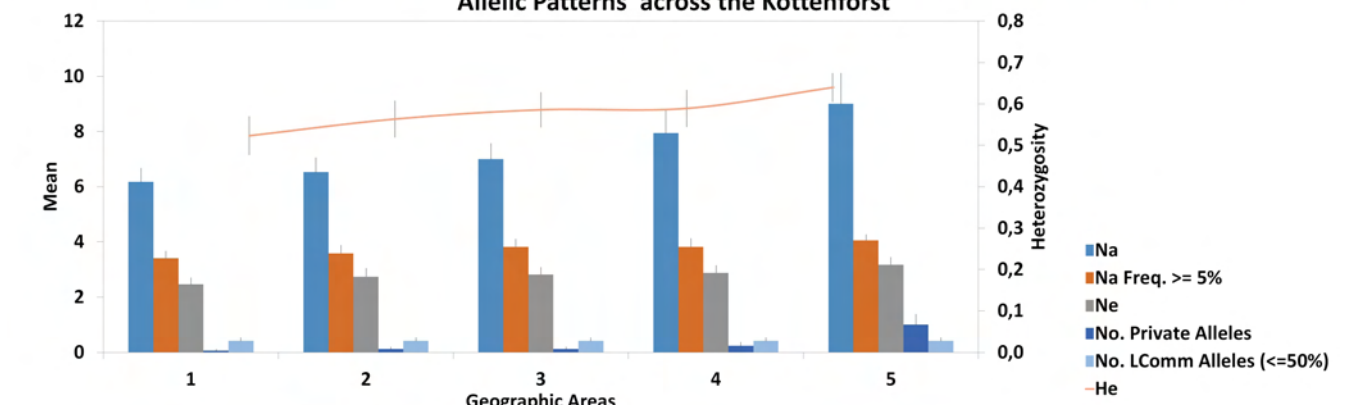
| Fst-Values	1	2	3	4	5
1	0.000				
2	0.008	0.000			
3	**0.009**	0.007	0.000		
4	**0.013**	**0.010**	0.006	0.000	
5	**0.017**	**0.014**	**0.009**	0.007	0.000

Supplementary Table 2: Data showing the genetic differentiation of salamander larvae by using Fst-values between 5 subareas (see Suppl. Fig. 2) relative to the total numbers of sampled larvae across the Kottenforst. Fst-values were calculated with the program Genalex 6.503. Significant Fst-values are in bold ($p \leq 0.05$).
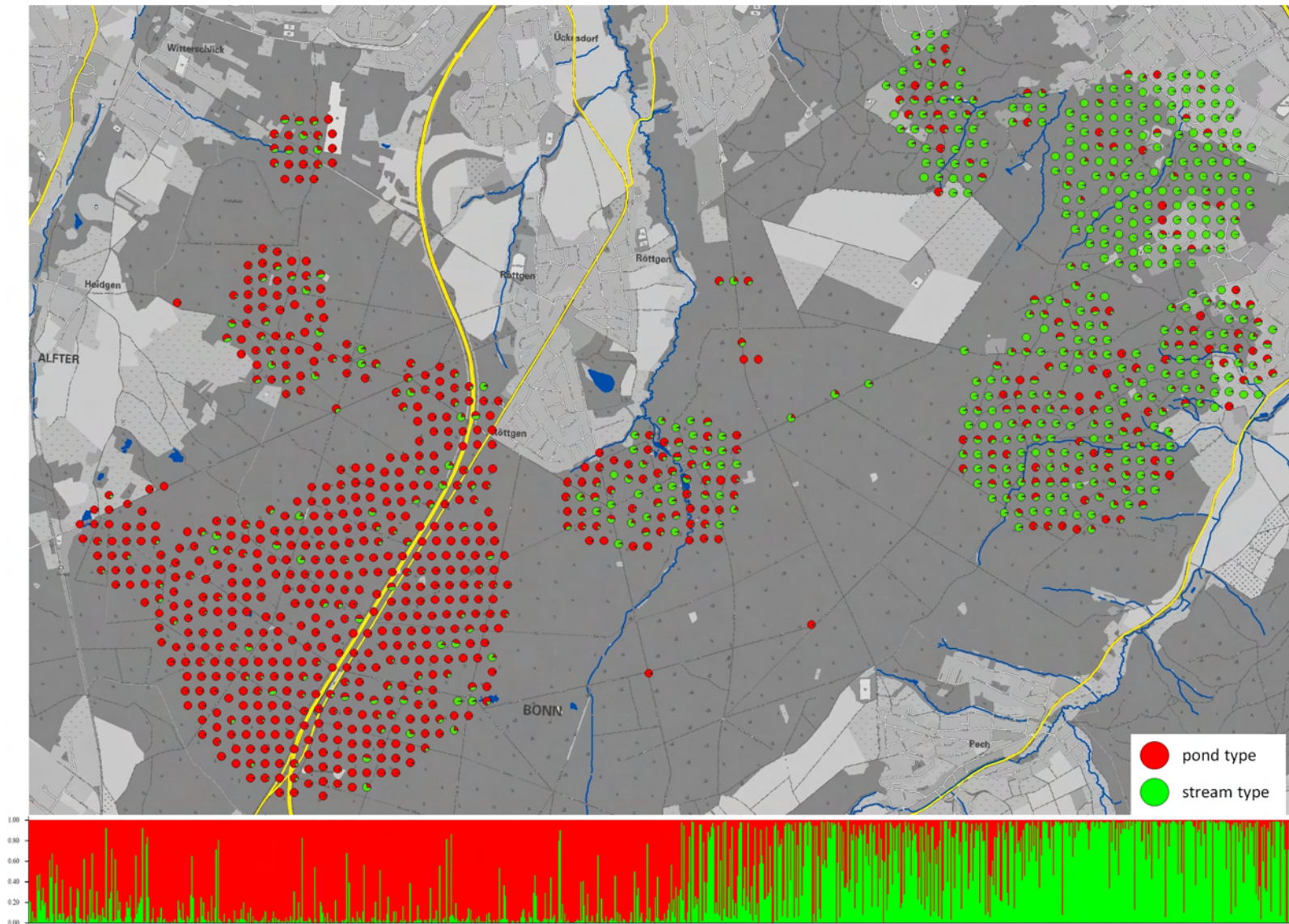
Supplementary Figure 4: Spatial distribution of 1106 adult genotypes across the Kottenforst for K=2 with STRUCTURE.
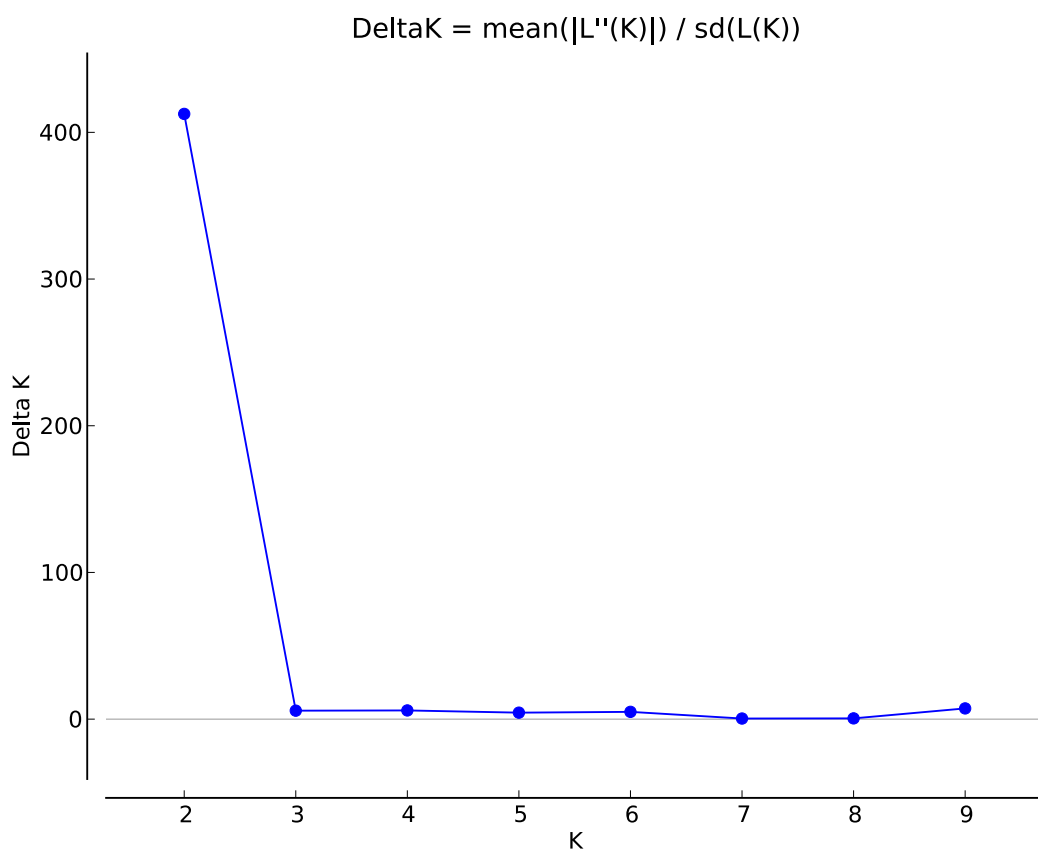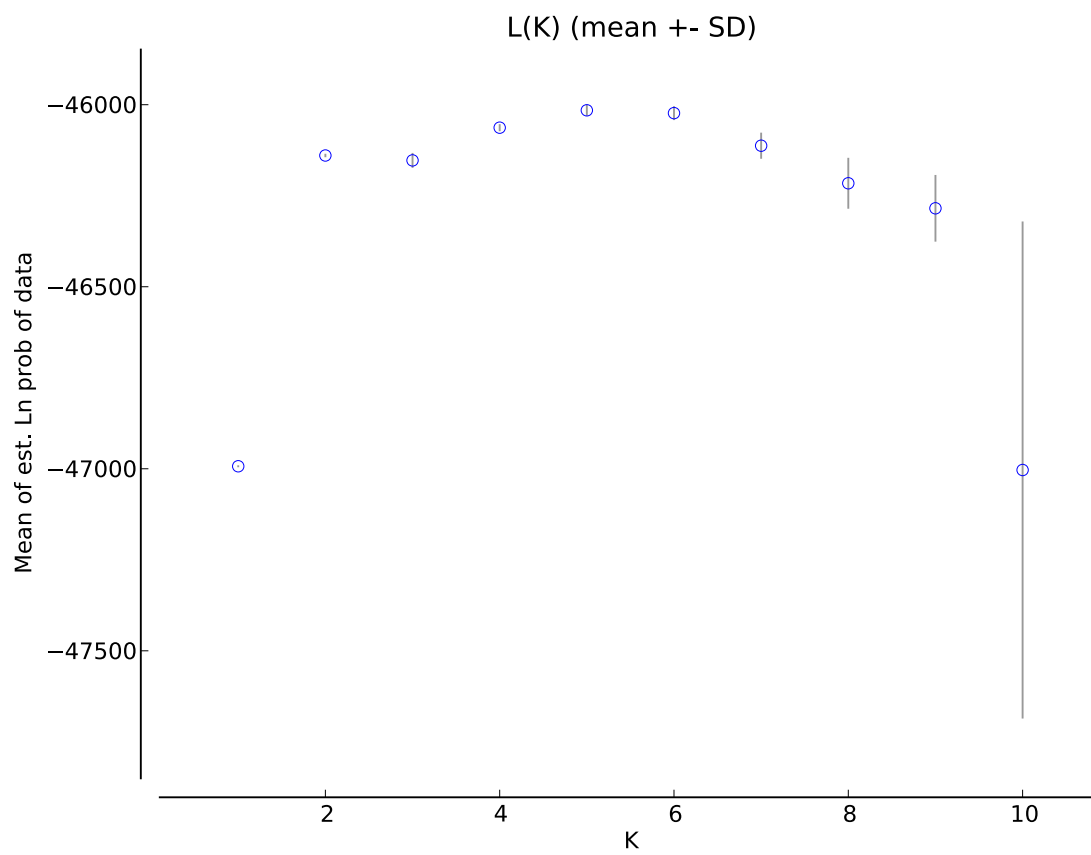
Supplementary Figure 5: Results of STRUCTURE HARVESTER for 1106 adult genotypes.
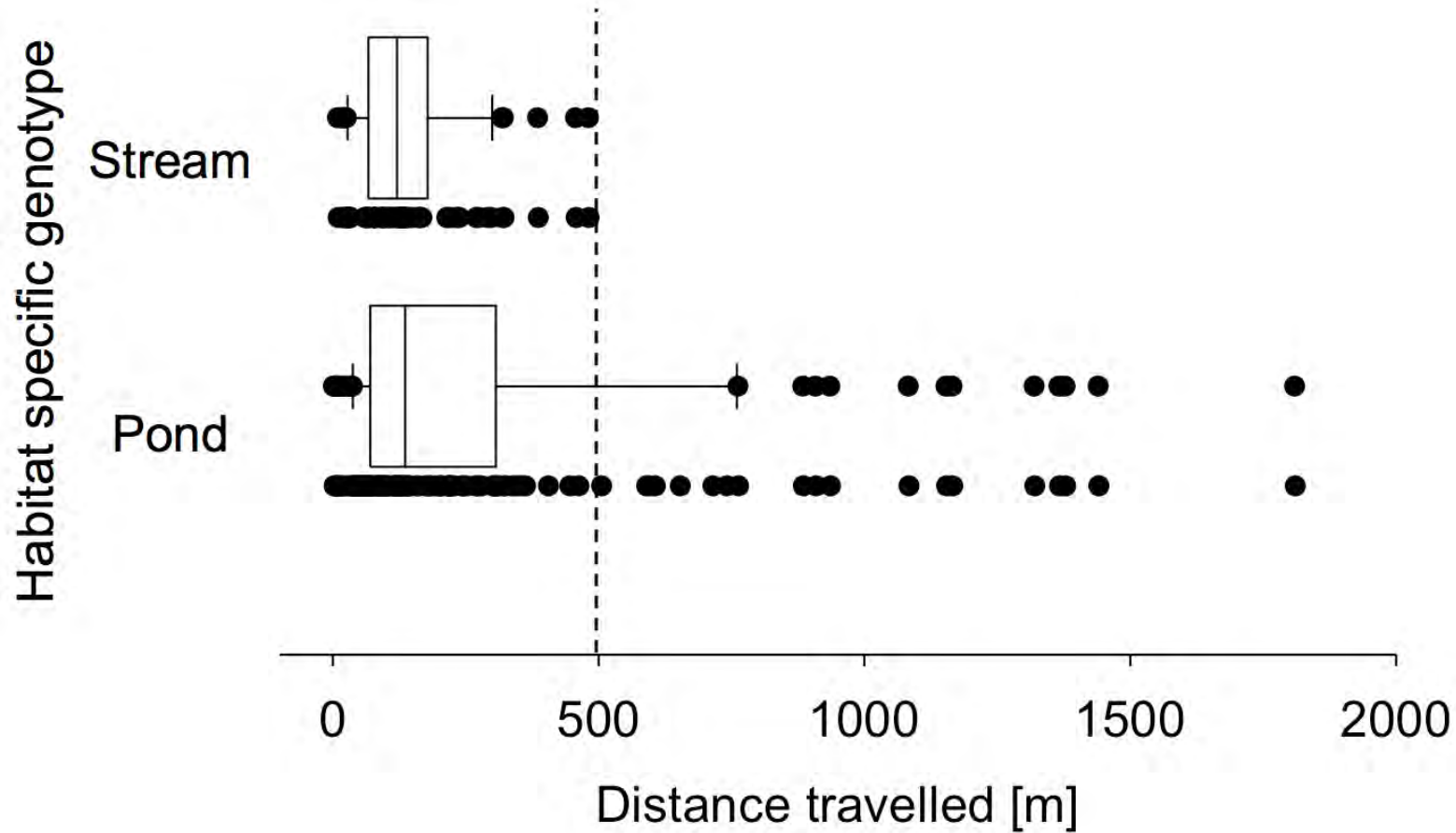
Supplementary Figure 6: Boxplot of moved distances of 178 recaptured adult individuals. Dots show individual data points, whereas the boxes indicate the 75%-percentile and the 25%-percentile, and the line in the box represents the median (i.e. 50% percentile).