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„Of active females and resting males: high inter-patch dynamics in a spatially structured population of *Hyalinobatrachium valerioi* (Centrolenidae)“

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Katharina Trenkwalder

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Für meine Eltern und meine Geschwister.

Hyalinobatrachium valerioi

Foto: Katharina Trenkwalder

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Of active females and resting males: high inter-patch dynamics in a spatially structured population of *Hyalinobatrachium valerioi* (Centrolenidae)

Key words Amphibians · Stream-associated breeder · Patchy population · Sex-biased dispersal · Patch connectivity · Between-patch dynamics

Introduction

The structure of a population can be seen as its response to the environment. Natural environments are usually not homogeneous; biotic and abiotic gradients differentiate the surface area into patches of favorable habitat which are intermixed from each other by unusable ones (Wiens 1976; Fahrig and Merriam 1985; Kotliar and Wiens 1990; May and Southwood 1990; Szacki 1999). 'Spatially structured populations' are composed of discrete patches of individuals (Revilla and Wiegand 2008) and are the typical response to patchy environmental conditions (Szacki 1999).

The survival of spatially structured populations is guaranteed by two components of population dynamics: intra-patch and inter-patch dynamics. Simplified, the former relates to survival and reproductive rates within each patch and the latter to the rate of movement and reproduction of individuals between patches (Wiens 1976; Fahrig and Merriam 1985; Revilla and Wiegand 2008). The dynamics of spatially structured populations are therefore the result of processes at the level of individuals and individual movement behavior can be translated to local population properties and then to population dynamics (Revilla and Wiegand 2008).

When between-patch dynamics break off (e.g. if changes in the environment restrict movement and thus gene flow between patches), small isolated populations

remain. Such small populations are more susceptible to genetic depletion through drift and inbreeding, with adverse consequences for viability (McKinney 1997; Beebee 2005). Therefore, conservation of spatially structured populations is of major importance, because they are at a higher risk of local extinction than non-patchy ones (McKinney 1997).

Amphibians represent the most endangered vertebrate class of the last decades with a dramatic global decline mainly in the last years (Stuart et al. 2004). Particularly in the Neotropics, which harbors nearly 50% of the worldwide amphibian species, more than half of the species are considered as “endangered” according to the IUCN (Urbina-Cardona 2008). Adequate conservation and management measures for amphibians are indispensable to preserve the genetic diversity and counter this rapid decline. The development of such measures requires the estimation of accurate census sizes as well as knowledge about population dynamics and population connectivity (Semlitsch 2000; Jehle and Arntzen 2002; Schmeller and Merilä 2007; Urbina-Cardona 2008). Furthermore, amphibian populations can serve as useful indicators for investigating the effects of climate change (cf. Carey and Alexander 2003; Corn 2005). Beside studies that involve long-term monitoring of current census sizes of amphibian populations, population genetic analyses are essential as they can provide a deeper understanding of population structure and dynamics. Molecular methods enable to identify kinship relationships and reconstruct whole pedigrees, thereby allowing to individually track the gene flow within and between populations (Blouin 2003; Urbina-Cardona 2008). However, only little is known about population connectivity in amphibian populations, and particularly for Neotropical frogs profound data on population dynamics at the individual level are lacking.

Glass frogs (Centrenolidae) are endemic in the Neotropics from southern Mexico to Brazil. All species of this family are nocturnal and their oviposition occurs outside the water on leaves overhanging water or on stones next to streams or waterfalls. After hatching, the tadpoles drop into the water where metamorphosis takes place (Cisneros-Heredia and McDiarmid 2007). A patchy distribution was detected for two studied populations in the glass frog species *Vitreorana aff. eurygnatha* (Gouveia et al. 2012) and *Hyalinobatrachium valerioi* (Vockenhuber et al. 2008).

Our study species *H. valerioi* occurs in Costa Rica, Panama, Columbia and Ecuador at tropical lowland streams (Savage 2002; Cisneros-Heredia and McDiarmid 2007; Kubicki 2007). In the wet season (from May to November) males call from the underside of leaves above streams to attract females. Clutches are deposited on the underside of leaves. After oviposition females immediately leave the breeding site. Males fertilize the eggs and provide parental care by permanently guarding their clutches until the larvae hatch. In that time they continue calling to attract more females, thus they may attend up to seven clutches of different developmental stages on the same single leave simultaneously (Savage 2002; Vockenhuber et al. 2008).

At our study area Vockenhuber et al. (2008) had determined a patchy distribution of calling *H. valerioi* males. According to this observation, to the parental behavior of *H. valerioi*, and to the fact that little movement is also seen in other glass frog males (*Centronella fleischmanni*, Greer and Wells 1980, Jacobson 1985; *Centrolene prosoblepon*, Jacobson 1985, Robertson 2008), we expected strong site fidelity and small action ranges for *H. valerioi* males in our study population. This high philopatry in males might lead to small exchange rates between patches and consequently reduce gene flow between patches.

Apart from the studies on male movement patterns in *C. fleischmanni* and *C. prosoblepon* (Greer and Wells 1980; Jacobson 1985; Robertson 2008), nothing is known about individual movement of females in this family. The reason for this gap of knowledge presumably relates to the rare captures of females (Robertson et al. 2008) due to their lack of site fidelity and cryptic (i.e. mute) behavior.

The aim of our study was to assess individual relatedness and between-patch dynamics of a spatially structured population of *H. valerioi*. To this end we integrated genetic and observational data, in order to get maximum information about population dynamics during our study period. Molecular tools should allow us to investigate patch connectivity, to reveal genetic relatedness of individuals across the entire population, and to perform parentage analyses. The latter should serve to reconstruct female mating activities and therefore give insights in female movement patterns. Focal observations in the field should help to gain a more concrete record of individual movements.

Material and methods

Study population and area

In this study we monitored a population of *H. valerioi* in the Esquinas rainforest near La Gamba, Costa Rica, at the tropical lowland stream 'Quebrada Negra'. The stream section where *H. valeroi* occurs during the reproductive season is located next to the tropical research station 'La Gamba' (8°42"61'N, 83°12'97"E). The southern stream bank is bordered by a secondary forest and the northern bank by the gardens of the 'La Gamba' field station. Downstream the landscape changes into an agricultural area which *H.*

valerioi does not use as habitat. Up to 300 m further upstream our study area *H. valerioi* could not be found. We defined a transect comprising a 425m long stream section including two small affluents (Fig 1).

Sampling of DNA and observational data

Sampling took place between August 15 2012 and November 23 2012, thereby covering almost the entire breeding season. We performed daily and nightly inspections – with the exception of 7 daily and 24 nightly inspections when the weather conditions made surveying impossible (e.g. too high stream water level after strong rainfalls). In the 101 days of our study period we performed 169 inspections summing up to around 300 hours in the field. The study transect was divided into 5m-sections. In nights we walked along the transect twice, up- and downstream, respectively, to compensate for temporal effects on observation probabilities of calling males and mating activity. Inspections started from 8 p.m. and lasted until no further new adults or individuals during courtship or mating were found. During daytime we controlled the site-presence of males seen the night before and checked the development of all recorded clutches. As this nocturnal species does not show calling, movement or mating activity by day, these surveys took place only one-way in upstream direction from around 10:30 a.m. to 11:40 a.m.

Males were found by acoustically locating their calls and by visually scanning the underside of leaves. In most cases females were detected at known male sites in amplexus or sitting next to a male during the daytime. Sexes were differentiated by calling activity and by presence/absence of eggs in oviducts visible through the skin (cf. Vockenhuber et al. 2008). Spatial locations of new as well as recaptured adults and clutches were recorded. We used 5-m section flags to estimate the position of observed

adult and clutch sites along the transect at 0.5 m precision. Previous studies at the study site have shown, that the average of *H. valerioi* oviposition sites can be expected at a height of 2.68 ± 1.12 m (range 0.2-6 m, n = 182) (Vockenhuber et al. 2008). By means of a ladder sampling and monitoring up to a maximum of 5 m above water surface was possible depending on vegetation and shore structure.

At each first capture event, we collected tissue samples of adults by clipping the third toe pad of both hind limbs for subsequent molecular analysis (cf. Ursprung et al. 2011). Females in amplexus were clipped only after oviposition. We took dorsal pictures on a scale paper for individual identification and later body size measurements (snout-urostyle length, SUL) with ImageJ (Abramoff et al. 2004). Immediately afterwards all individuals were released at the site of first observation. Redetected individuals which could be recognized by their missing toe pads were not caught again; dorsal pictures were taken while sitting on site. For simplicity, from now on the term 'recapture' is used for this redetection events although the individuals were not re-caught in a literal sense. These pictures were then matched visually with the photo database of previously recorded individuals. We also took pictures of clutches to identify the number of larvae and recorded their developmental stage. For offspring tissue we sampled two well-developed larvae per clutch (at least Gosner stage 17; Gosner 1960), if the clutch was very small (less than 10 larvae), only one tadpole was taken. All tissue samples we put into prepared labeled tubes with 96% ethanol.

DNA extraction and microsatellite genotyping

Total genomic DNA was isolated by Proteinase K digestion according to a standard phenol-chloroform protocol (Sambrook et al. 1989). DNA of each individual was

amplified at eight microsatellite loci (*Hyval04, Hyval10, Hyval16, Hyval17, Hyval20, Hyval21, Hyval22* and *Hyval24*) using respective PCR primers and protocols according to Ringler et al. (2014). The amplified products were diluted with water and mixed with internal size standard ROX350 to run on an ABI 3130x1 sequencer and be analyzed using PeakScanner 2.0 (Applied Biosystems). We identified all loci visually and determined the final allele size using the binning software TANDEM v1.08 (Matschiner and Salzburger 2009).

Parentage analyses

Parentage assignment was conducted with COLONY 2.0 (Jones and Wang 2010), a likelihood based method. We implemented the full likelihood model with medium precision, without sibship prior, allowing polygamous mating in both sexes. COLONY provided a simulated parental genotype if a parent of our genotype dataset could not be assigned. We exclusively used assignments with 'Best (ML) Configuration' and the maximum likelihood (cf. COLONY user guide). For visualizing the mating network along the stream we used the program NodeXL (Smith et al. 2010).

Analyses of population size and structure

The effective population size N_e was estimated by COLONY 2.0 full likelihood method assuming random mating. The sampling coverage was determined by estimator MMMeans using the program EstimateS 8.2.0 (Colwell et al. 2004). We calculated the fixation index F_{st} with FSTAT 2.9.3.2 (Goudet 2001) to analyze the genetic connectivity between the patches and used the program KINGROUP v2 (Konovalov et al. 2004) to determine the pairwise relatedness coefficients *r* (Queller and Goodnight 1989) between all pairs of adult individuals. The coefficient *r* describes a continuous measure of overall

genetic similarity between two individuals within a population. The overall performance of *r* is expected to increase with the coverage of sampling and to be even accurate using low numbers of loci with few alleles (Konovalov and Heg 2008). Values of this coefficient range from -1 to +1; the higher *r* the higher is the probability of recent coalescence than random pairs within the population, and reciprocal (Queller and Goodnight 1989; Blouin 2003; Konovalov and Heg 2008). To get reference values of *r* for full and half sibs we used the simulation function of KINGROUP v2, which estimated the expected relatedness among 100 full siblings, 100 half siblings and 100 'unrelated' individuals, based on the allele frequencies of our genotype data. Values for pairwise relatedness are expected to be on average 0.5 for full sibs, 0.25 for half sibs and zero for the population mean (Konovalov and Heg 2008; Blouin et al 1996). Furthermore we used the *r* values to detect adult full and half sibs across our study population. To display the distribution of male full siblings according to their respective patches we used the program NodeXL.

Analyses of individual action ranges

We defined 'action range' as the distance between the two most distant sightings of one individual along the transect and 'total movement' as the sum of the distances between consecutive sightings per individual. Single sighted individuals are therefore not considered in our calculations. For males we can equate 'action range' with 'home range' because of our high number of observational data points per individual. Due to the low recapture rate of females, we also included female clutch sites according to the results of our parentage analyses in addition to observational data points, to calculate their action range and total movement. Also simulated females with multiple clutches

got assigned values for action range and total movement. We conducted a Mann-Whitney-*U*-Test to test for differences in the action ranges between males and females. Possible influences on individual action ranges or total movement were checked by pairwise Pearson correlations: We tested for relationships between action range, total movement and SUL. For females we further checked if there is a relationship between total movement and average number of eggs, as higher number of eggs might cause a reduction in mobility. Additionally we evaluated the spatial distances between two consecutive clutch deposition sites per females. We only included clutches with known deposition dates in these analyses.

Results

In the period between August 15 2012 and November 23 2012 we sampled in total 143 adults (93 males, 48 females, 2 sex undefined) and 374 larvae out of 193 clutches.

Parentage

With the exception of two clutches, every detected clutch was guarded by a male. We discarded 35 larvae of 19 different clutches from further analyses due to low amplification success after up to three times re-genotyping. Individuals with ambiguous sex were excluded from all sex-based analyses. We used the software COLONY to perform parentage analysis for all remaining 339 larvae (174 clutches). 216 larvae (111 clutches) could be assigned to both a known father and a mother in our study population. For the remained 123 larvae (63 clutches) the assigned father was within our sampled genotypes, while the mother was not, as simulated genotypes were assigned by the program to these larvae. In 20 cases (40 larvae) larvae from the same clutch –

presumably full siblings – were assigned to the same known father but different simulated mothers (we excluded the possibility for multiple maternity), we reanalyzed all larvae for which their mother was not sampled in pre-defined full sib groups. Altogether 30 female genotypes were simulated.

Population size and structure

The study area contained aggregations of reproductively active frogs and sections where no individuals or clutches could be found. We arbitrarily separated these spatially clustered breeding aggregations into distinct patches. We defined a patch as those groups of individuals that were separated from each other by a maximum of 25 m; thereby we identified three different sized patches along our study transect. Patch 1 started 20 m after the zero point of our transect and extended over 135 m in upstream direction. It was separated by 33.5 m from patch 2 which had a length of 81.5 m. Between patch 2 and patch 3 was a non-breeding section of 51.5 m. Patch 3 with a length of 90.5 m ranged up to 12.5 m before the end of the transect and included sections of two small affluents (Fig. 1). Patch 1 consisted of 48 males, patch 2 of 16 and patch 3 of 28. Only one male was detected in more than one patch. This male moved from patch 1 to patch 2 within our study period. It was reproductively not successful and therefore not considered in all our patch-based analyses. The patchy distribution of the males along the transect over the study period is given in Figure 2. The females could not be assigned to specific patches, due to their high movement activity (see results of individual action ranges below).

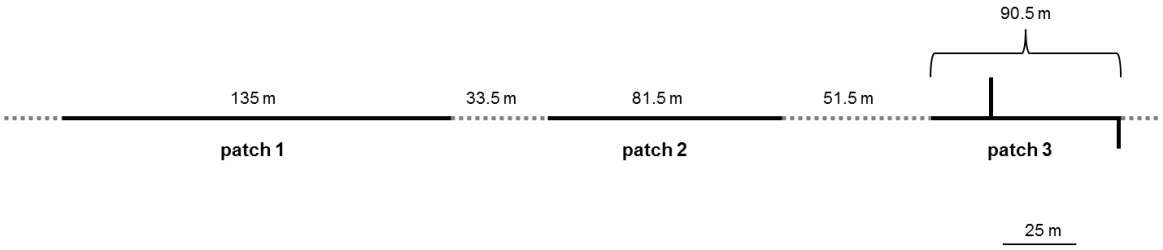

Fig. 1 Patchy population structure of the *H. valerioi* under study. Sections where no individuals were detected within 25 m are given as dashed lines; patch 3 included two small affluents. The natural stream course is not considered in this graph.

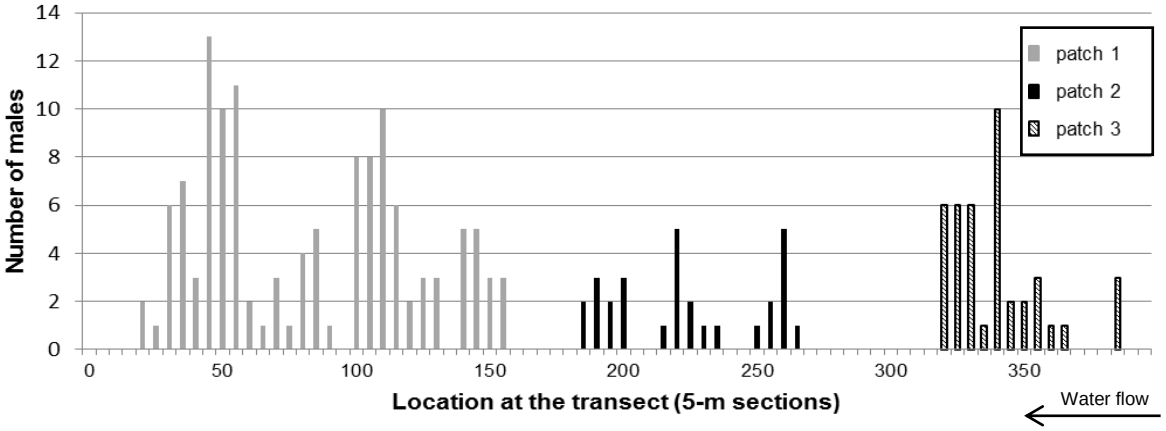

Fig. 2 Distribution of *H. valerioi* males along our study transect during the study period. Shades of columns correspond to different patches; multiple sightings of single individuals within a 5 m-section are not considered in this graph.

The recapture rate of males within the study period was on average 26.3 sightings per individual (median = 21, range = 1-99), while females hardly were sighted more than once (average = 1.9, median = 1, range = 1-6). From this low recapture rate no MMMeans estimator was calculated for the females. Based on the male recapture rate the estimator MMMeans determined a number of 93 males for the population. COLONY estimated the effective population size N_e to be 131.

The analyses of genetic differentiation revealed a high genetic connectivity between the patches (F_{st} over all loci = 0.002). We then analyzed the genetic setup of the population by calculating relatedness values between all possible pairs of individuals. We first used the simulation function in the program KINGROUP to calculate average pairwise relatedness of full siblings (r ± SD = 0.4694±0.1672), half siblings (r ± SD = 0.2462±0.1511), and 'unrelated' individuals (r ± SD = -0.0159±0.1367) based on the actual allele frequencies in our population (Fig. 3). Based on these estimates, we identified 198 full sib (r > 0.35) and 2006 half sib (r = 0.10-0.35) relationships distributed over the whole population. Among the full sibships were 79 male pairs, 24 female pairs, 88 mixed pairs and seven pairs where at least for one individual the sex was undefined. 50.6% of male full siblings were found across different patches, 48.1% within the same patch. One full sib pair was detected between the male that changed patches during our study period and a male of patch 1. The distribution of male full siblings across patches is illustrated in Figure 4.

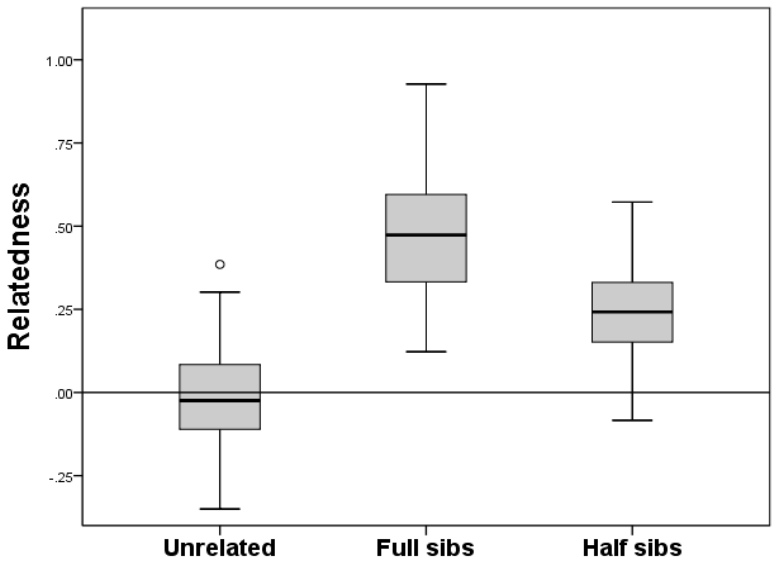

Fig. 3 Pairwise relatedness of the KINGROUP simulations. The boxplots display the distribution of pairwise relatedness values in the KINGROUP simulations ('unrelated', 'full sibs', 'half sibs').

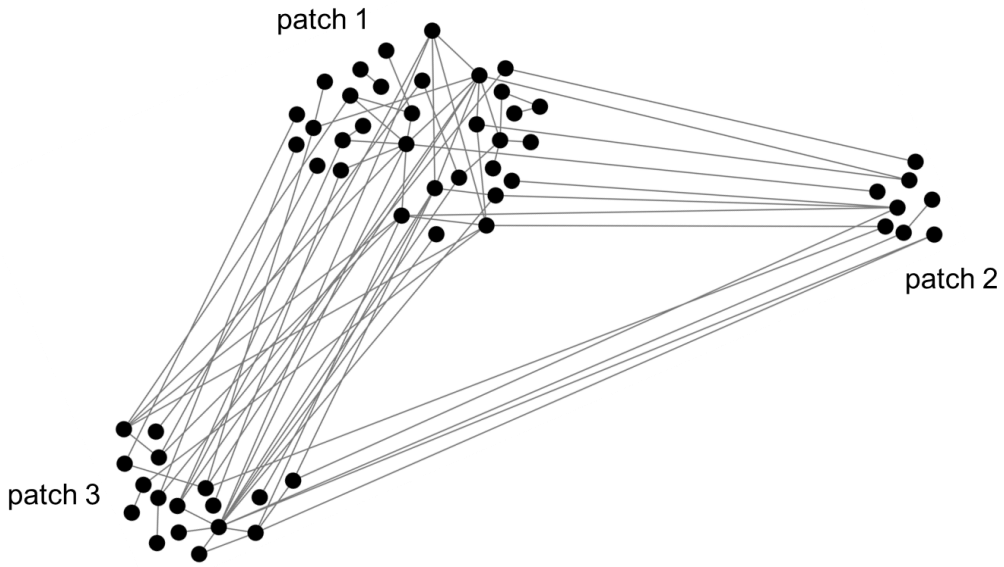

Fig. 4 Distribution of male full siblings across population patches in *H. valerioi*. Males are illustrated by dots and attributed to their respective patch; full sib pairs are connected with a line. The sibship of the male that changed patches during our study is not displayed in this graph.

Individual action ranges

The action range was significantly higher in females than in males (m/f: n = 79/57, median = 8/17 m, range = 0 – 84.5/162.5 m; Mann-Whitney: U = 1518, P = 0.001; Fig. 5).

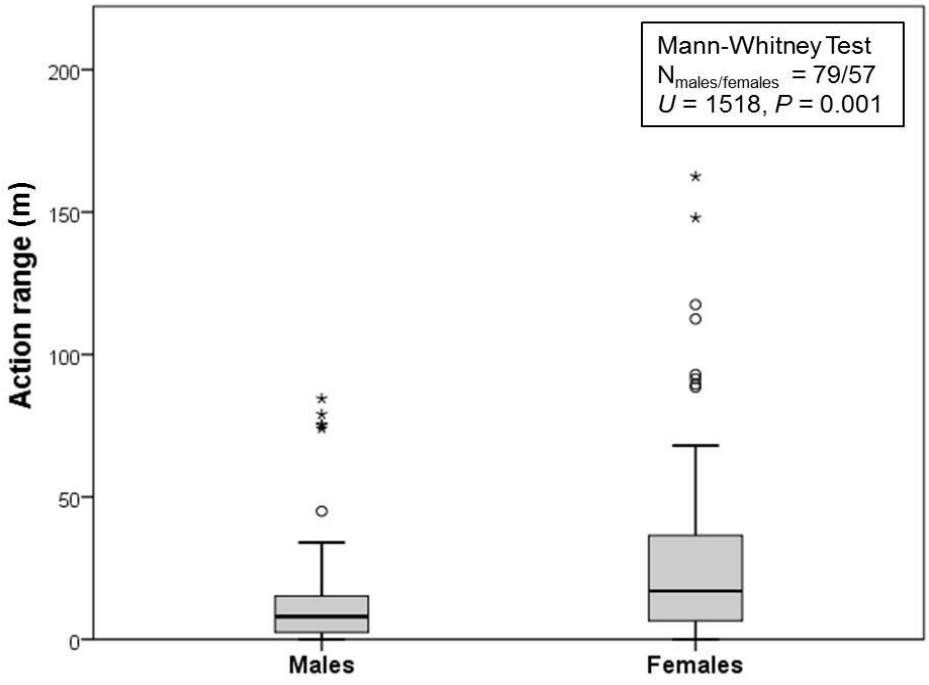

Fig. 5 Boxplots of action ranges in male and female in *H. valerioi*.

Total movement of the females was also much higher in females (m/f: n = 79/57, median = 15/22.5 m, range = 0 – 139.5/405.5 m). We found a significant positive relationship between action range and total movement in males as well as in females (m: r = 0.943, P = 0.000, n = 79; f: r = 0.951, P = 0.000, n = 57). The high female action range along the stream is illustrated in the mating network (Fig. 6). This graph also illustrates that seven females mated with males that were located in different patches.

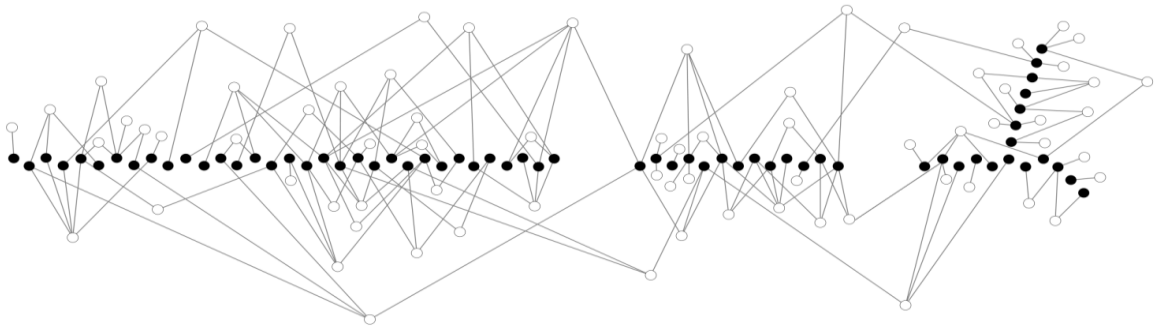

Fig. 6 Mating network of the *H. valerioi* population under study. Males (black dots) and females (white dots); order of males displays their relative order of occurrence along the stream; the third patch includes two small affluents; distances in the graph do not correspond with absolute distances in the field.

However, the distance between two consecutive clutch deposition sites of a female is in 65% within a maximum of 15 m (Fig. 7). We did not find a significant relationship between total movement and SUL in both sexes (m: r = 0.022, P = 0.885, n = 44; f: r = -0.274, P = 0.175, n = 26). For females there was also no significant correlation between total movement and average number of eggs (r = -0.178, P = 0.193, n = 55).

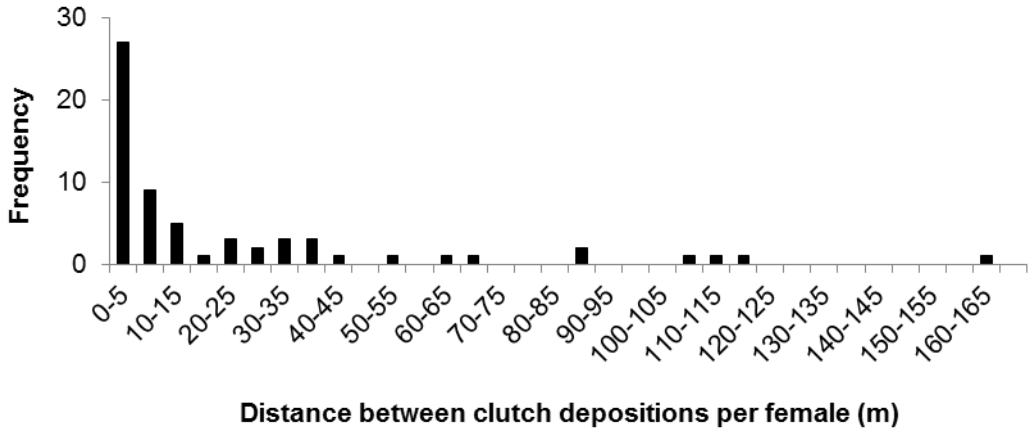

Fig. 7 Frequency distribution of the distance between consecutive clutch deposition sites per female in *H. valerioi*.

Discussion

In our study population *H. valerioi* males showed small action ranges and high site fidelity, and occurred in a patchy distribution along the stream – creating a so-called 'spatially structured population'. The females, by contrast, were moving over large distances within and between those male aggregations to find their mates for reproduction. Our data revealed that there is high genetic inter-patch connectivity and closely as well as unrelated males were equally distributed over the whole population.

Parentage analyses

We performed parentage assignments for our sampled offspring using microsatellite markers. The results of the genetic parentage analyses corroborated our observational field data in nearly all cases. Genetic paternity was predicted correctly for 171 of 174 analyzed clutches. This underlines the efficiency of individual identification through dorsal color pattern in this species. However, genetic parentage assignments were particularly valuable for the identification of maternity. For 23% of the analyzed clutches female oviposition events could be observed directly and were confirmed by the genetic analyses; for the remaining 77% clutches, genetic parentage assignments were indispensable. In almost all cases, larvae sampled out of same clutch were independently assigned to the very same fathers and mothers (n = 101). However, in 20 cases larvae out of same clutches got assigned equal known fathers but different simulated mothers. We attribute these assignments to be due to small genotyping errors as multiple maternity was excluded by us because of the reproductive behavior in *H. valerioi*. Therefore we decided to reanalyze all larvae for which not a single maternal genotype was assigned in pre-defined full sib groups.

The advantage of parentage analyses in our study was that it opened up a way to reconstruct female movement by revealing their breeding sites. The assessment of movement patterns in both sexes is important to make real predictions about population connectivity (Robertson et al. 2008). The restriction to movement to only parts of the population (e.g. only males) might lead to biased conclusions about general population dynamics (cf. Loewe 2003). Thus we aimed to record both, male and female movements. Our detected female action ranges and total movements are minimum estimates. On one hand, a few clutches were not reachable, 11% out of all recorded clutches (n = 217) could not be sampled due to clutch mortality (e.g. fungal infection, predation) before tadpoles reached the minimum developmental stage required for sampling, and 19 clutches with incomplete genotypes had to be discarded from parentage analyses. On the other hand, we hardly obtained information about movement of females that did not involve any mating activity. Nevertheless, by the reconstruction of breeding activities we could gain insight into actual patterns of gene flow within our study population.

Population size and sampling coverage

Hyalinobatrachium valerioi populations have the highest mating activity when the rainfall maxima of the rainy season are reached (Kubicki 2007; Vockenhuber et al. 2008). Highest precipitation in our study area is from August to November (Weber et al. 2001). By monitoring our study population from August 15 to November 23 we covered the entire main breeding period in the year 2012.

Every analysed larvae could be assigned to a male that was actually sampled by us. Even the two clutches where no guarding male was detected were assigned to

known males; no single father was simulated. These facts indicate a high sampling coverage of males. This is also corroborated by the MMMeans estimate of males (MMMeans = 93) which was equal to our actual sample size N_{males}. We therefore assume having sampled all reproductively active males in our study transect. The MMMeans is a sample-based rarefaction estimator usually used for species richness calculations but also workable for estimating census size in 'closed' populations over a long observation period by calculating asymptotic population size estimates based on Michaelis-Menten kinetics (cf. Ursprung et al. 2011). The fact that *H. valerioi* occurs neither up- nor downstream of our study transect and is a prolonged stream-associated breeder that stays at the stream during its breeding season allowed us to treat the individuals in our study transect as a confined reproductive community. As *H. valerioi* females were rarely recaptured, it was not possible to estimate their census size based on the MMMeans calculations. However, we had a detection size of 78 females, where 48 were actually sampled by us and additional 30 female genotypes were simulated by the COLONY analysis. As we were not able to sample all clutches and thus presumably also could not discover every female, we expect the real census size of the females to be higher than this number, which will result in an equal sex ratio in our study population. This conclusion is also supported by the results of Mangold (2014) who detected a similar opportunity for sexual selection in males and females and a sex ratio of 0.9 males per female in actual reproducers of our *H. valerioi* population. An unbiased sex ratio for our study population is also corroborated by the high effective population size: with a N_e of 131 and an adult number of N = 173 (93 males, 78 females) the N_e/N ratio in our study population is with a value of 0.76 well-balanced. This N_e/N ratio further indicates that the reproductive success in this population is high (cf. Hartl and Clark 1997; Primack

2006). Reproductive success is a major component of fitness and can be defined as the relative production of fertile offspring by a genotype, thus reproductive potential and offspring survival to maturity (Crognier 2003). A study on the mating system of our *H. valerioi* population revealed that 69% of adult males had mating success within one breeding season (Mangold 2014). Equally high success rates can also be assumed for the females because females are actively approaching calling males and according to Vockenhuber et al. (2008) 93.3% of the amplexi in *H. valerioi* are successful. The large number of 198 full siblings indicates a high rate of reproductive success in our study population.

Patchy distribution of males

The *H. valerioi* population under study was composed of three distinct patches (Fig. 1), with only the patchy distribution of the males leading to this population structure. With the exception of one single individual, every male stayed in the patch of its first sighting during the whole study period. The male reproductive behavior of clutch guarding on the one hand, and female choice on the other hand, can be interpreted as factors that favor male low action ranges and high site fidelity, which in turn hold these patches stable. Spatial stability of the patches is even observed inter-seasonally (Trenkwalder et al. in prep.).

There are several possible factors that might have led to such a patchy distribution of males. These patches might be resource-based and a result of males gathering at suitable breeding habitat (Wells 1977). However, we did not identify any obvious differences between used and unused stream sections. Also in other anuran species aggregated male choruses appear concentrated at only parts of water habitats

while they do not use surrounding areas of equal quality (e.g. *Hyla regilla*, Whitney & Krebs 1975). The resulting chorusing of male frogs is much more attractive to approaching females than single calling males (Ryan et al. 1981). Another possibility would be spatial separation from other local glass frog species in order to avoid competition, as already suggested by Vockenhuber et al. (2008). Further investigations by e.g. monitoring of all five local glass frog species (Vockenhuber et al. 2008), comparison of vegetation, food availability or predator presence in breeding and non-breeding sections, male-removal experiments out of patches or chorus-simulations through playbacks are needed to investigate reasons for the spatial clustering in this *H. valerioi* population.

Aside from these two factors this patchy distribution could also display the existence of defined reproductive communities. The relatively small distances between patches in our population of *H. valerioi* do not necessarily imply that there is high genetic connectivity between them. Genetic differentiation over small distances like 50 m in frogs was e. g. detected in *Geocrinia rosea* (Driscoll 1998). However, the calculated F_{st} values for our study population indicated low genetic differentiation between the patches. This was also corroborated by the finding that females mated with males from different patches and male full siblings were found across the entire population (Fig. 4). The patches therefore do not relate to defined reproductive communities. Similar results were reported in a study on another glass frog species, *C. prosoblepon*, where genetic homogeneity of stream subpopulations within a 1.2 km^2 study area was found (Robertson et al. 2008).

High female action ranges

We did not assign females to specific patches, because our investigations revealed that they have a significantly higher action range than males (m/f: n = 79/57, median = 8/17 m, range = 0 – 84.5/162.5 m; Fig. 5) and display neither site nor patch fidelity. Indeed, most females moved more or less long distances within a patch and nine females also moved between patches (Fig. 6). Three females even changed twice between two patches within the breeding season. The significant correlation between action range and total movement indicates that females do not permanently change between up- and downstream moving direction but show in general quite one-sided directed movement patterns. Although the distance between two consecutive clutch deposition sites was rather small (Fig. 7) the total movement in the course of the entire breeding season was high (median = 22.5 m, range = 0 – 405.5 m; within a transect length of 425m),

A sex-biased dispersal as detected for this *H. valerioi* population is a common pattern in birds and mammals; in many species one sex is more philopatric than the other. The benefits of sex differences in dispersal are reproductive benefits through increased access to mates or resources and the avoidance of inbreeding (Greenwood 1980). Little is known about dispersal asymmetry in amphibians but expected for several amphibian species; e.g. female-biased dispersal was detected in the bullfrog *Rana catesbeiana* (Austin et al. 2003). In our study population high female movement presumably did not affect genetic benefits, given that genetic relatedness and spatial distribution of males were not linked. Male full and half siblings were uniformly distributed over the entire population (Fig. 4) and there was no appreciable genetic difference between the discrete patches. However, apart from genetic benefits, moving

over large distances could also hold other advantages for female reproduction. Variation in habitat structure could result in breeding areas of variable quality: spatially distinct egg deposition sites with different conditions may affect clutch survival and mortality. Mortality of *H. valerioi* clutches is documented by predation events, physical destruction or altered exposure of oviposition sites and fungal growth (Vockenhuber et al. 2008; Vockenhuber et al. 2009). Consequently, depositing clutches at distinct sites could have been advantageous for the females in sense of spreading the risk of offspring mortality (cf. den Boer 1968).

High inter-patch dynamics

Genetic connectivity of distinct patches in spatially structured populations is maintained by between-patch dynamics; as long as there is an immigration rate of minimum 10% per generation, patches within populations are assumed as not independent (Hastings 1993). In our *H. valerioi* population we detected that females were able to overcome long distances (action range: median = 17 m, range = 0 – 162.5 m; total movement: median = 22.5 m, range = 0 – 405.5 m; within a transect length of 425m) (Fig. 5). Seven of the 9 females which were observed in multiple patches also mated with males of different patches (Fig. 6) and thus were conductive to inter-patch gene flow; this is equivalent to 13.2% of all females with multiple clutches (n = 53). Therefore moving long distances cannot only be seen as a personal advantage for the female as described before, but also as a mechanism to maintain genetic population connectivity.

We detected one male moving between patches that changed in upstream direction from patch 1 to patch 2 within our study period. Within 15 recapture events this male was observed sitting at the leave of another calling male or sitting/calling from

the upper side of leaves, which is uncommon for this species (Vockenhuber 2008). Its body size did not differ from other males, which might have been an indication for a juvenile individual. We assume that this male did not have or recently lost his territory and was searching for a new one. However, it was reproductively not successful during our study period, suggesting that males probably change calling sites when they are not successful in attracting females.

Furthermore we detected large numbers of male full and half siblings located in different patches (Fig. 4). This pattern could be the result of either the spreading and dispersal of larvae, of metamorphs, or of the movement of adult males. As centrenolid tadpoles are fossorial and burrow into gravel or under leaves in the streams (Wells 2007), active movement of larvae over long distances seems rather unlikely. Inter-patch movement of such a large number of male adults is in contrast to our observations of high male inter- and intra-seasonal site fidelity (this study; Trenkwalder et al. in prep.). Consequentially, only movement of metamorphs remains as the most likely explanation for the observed uniform distribution of male full siblings across the population patches. Juvenile dispersal has been shown to be an essential factor for population connectivity in amphibians (as reviewed in Cushman 2006). Therefore, future studies should focus on individual juvenile movement patterns after metamorphosis in *H. valerioi*.

Conclusions and prospects

In general, understanding movement patterns is essential to be able to make predictions about actual patterns of gene flow in riparian frog species (Lowe 2003; Funk et al. 2005), species recolonization potential (Robertson et al. 2008), and to develop suitable conservation measures. While Costa Rican populations of *H. valerioi* are assumed to be

stable, *H. valerioi* is supposed to be in decline in Panama (IUCN 2006). Furthermore, other glass frog species are listed as endangered or vulnerable, and for many populations a current status cannot even be defined due to data deficiency. With this study, new insights in population structure, dynamics and connectivity of glass frogs are provided: We could reveal a spatially structured population of *H. valerioi*. The patchy distribution of the philopatric, little moving adult males led to this population structure. The detected a high genetic connectivity between these patches was likely maintained by two different components of inter-patch dynamics: dispersal of metamorphs and high mobility of reproductively active females.

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Abstracts

English abstract

'Spatially structured populations' are the typical response to patchy environmental conditions. When inter-patch dynamics break off, small isolated populations remain which are more susceptible to genetic depletion through drift and inbreeding, resulting in a high risk of local extinction. Precise knowledge about mechanisms of population dynamics is indispensable to provide effective conservation measures for such populations. Amphibians which currently have to face a dramatic global decline represent the most endangered vertebrate class on earth. Little is known about population connectivity in amphibian populations, and particularly for Neotropical frogs profound data on population dynamics at the individual level are lacking. We monitored a patchy population of the glass frog *Hyalinobatrachium valerioi* at the tropical lowland stream Quebrada Negra near La Gamba, Costa Rica, during their reproductive season. We recorded precise spatial locations of all frogs and clutches, and collected tissue samples from the adults and clutches for molecular parentage analyses with highly polymorphic microsatellite loci. We used an integrative approach of observational field data and molecular analyses to investigate inter-patch dynamics and genetic population connectivity. Along a 425 m river transect we identified three spatially clustered breeding aggregations of philopatric, little moving males that showed little genetic differentiation. This high genetic connectivity between the patches was likely maintained by two different components: random dispersal of metamorphs and high mobility of reproductively active females.

Deutsche Zusammenfassung

‚Räumlich strukturierte Populationen' können als eine Antwort auf uneinheitliche Umweltbedingungen gesehen werden. Wenn die Dynamiken zwischen Aggregationen einer solchen Population abreißen, bleiben kleine isolierte Populationen übrig. Diese sind sehr anfällig für genetische Verarmung durch genetische Drift und Inzucht und damit einem hohen Aussterbensrisiko ausgesetzt. Genaue Kenntnis über Mechanismen von Populationsdynamiken ist daher unabdingbar, um effektive Schutzmaßnahmen für räumlich strukturierte Populationen entwickeln zu können. Amphibien müssen derzeit einen dramatischen globalen Rückgang erfahren. Sie repräsentieren die am höchsten vom Aussterben bedrohte Gruppe der Wirbeltiere. Bisher ist über Konnektivität innerhalb von Amphibienpopulationen noch wenig bekannt, gerade für neotropische Frösche fehlen Daten über Populationsdynamiken auf individueller Ebene. Wir haben eine räumlich strukturierte Population des Glasfroschs *Hyalinobatrachium valerioi* am tropischen Tieflandstrom Quebrada Negra in der Nähe von La Gamba, Costa Rica während seiner Reproduktionsperiode untersucht. Dazu haben wir exakte räumliche Positionen von allen Fröschen und Gelegen aufgenommen und Gewebeproben von Adulttieren und Gelegen gesammelt, um anschließend molekulare Elternschaftsanalysen mithilfe von hoch polymorphen Mikrosatellit Loci durchzuführen. Um die Dynamiken zwischen den einzelnen Aggregationen und die genetische Konnektivität innerhalb der Population aufzudecken zu können, haben wir uns einer integrativen Herangehensweise bedient und haben dabei Beobachtungsdaten mit molekulare Analysen kombiniert. Wir haben drei räumlich getrennte Fortpflanzungs-Aggregtionen entlang eines Bach-Transektes identifiziert, welche von philopatrischen, wenig dislozierenden Männchen gebildet worden sind. Zwischen den einzelnen Aggregationen haben wir keine

nennenswerten genetischen Unterschiede aufdecken können. Diese hohe Konnektivität zwischen den Aggregationen wird vorraussichtlich durch zwei verschiedenen Komponenten aufrechterhalten: willkürliche Verteilung der Metamorphlinge entlang des Baches und hohe Mobilität reproduktiv aktiver Weibchen.

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Curriculum Vitae

N A M E

Katharina Trenkwalder

S C H U L A U S B I L D U N G

09/1995 – 07/1999	Grundschule Krankenhausstraße, Garmisch-Partenkirchen
09/1999 – 06/2008	Werdenfels-Gymnasium, Garmisch-Partenkirchen Allgemeinen Hochschulreife (27.06.2008), Notendurchschnitt: 1,7
10/2008 – 07/2009	Tourismusmanagement FH München
10/2009 – gegenwärtig	Lehramtsstudium UF Biologie und Umweltkunde UF Deutsch, Universität Wien

S T U D I E N B E Z O G E N E A U S L A N D S A U F E N T H A L T E

08/2012 – 09/2012	Feldarbeit für Diplomarbeit in La Gamba, Costa Rica
10/2013	Feldarbeit für Glasfrosch-Wiederfangstude in La Gamba, Costa Rica
02/2014	Projektpraktikum „GIS-Feldmethoden und Verhaltensökologie & Diversität neotropischer Amphibien“ der Universität Wien in der Forschungsstation Pararé, Französisch Guyana

S T U D I E N B E Z O G E N E B E R U F S E R F A H R U N G E N

12/2003 – 04/2005	freiberufliche Skilehrerin für Kinder und Jugendliche in der Skischule Garmisch (Qualifikation „Skilehrer Grundstufe Alpin“)
01/2003 – 03/2007	Skitrainerin für Kinder und Jugendliche im Skiclub Garmisch

B E S O N D E R E A U S Z E I C H N U N G E N

01/2012	Leistungsstipendium der Universität Wien
06/2012	Förderungsstipendium der Universität Wien
01/2013	Leistungsstipendium der Universität Wien
01/2014	Leistungsstipendium der Universität Wien

PRÄSENTATIONEN & PUBLIKATIONEN

05/2013 — 29. Jahrestagung der österreichischen Lateinamerikaforschung (Strobl am Wolfgangsee, Österreich) – mündliche Präsentation: „Glasfroschforschung am Quebrada Negra, Costa Rica – genetisches Paarungssystem und Konnektivität von (Meta-) Populationen von Hyalinobatrachium valerioi“

08/2013 — 17th European Congress of Herpetology (Veszprém, Ungarn) – Posterpräsentation: „Of active females and resting males – High (Meta-) population connectivity *in Hyalinobatrachium valerioi*“

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PERSÖNLICHE FERTIGKEITEN/KOMPETENZEN

Sprache: Deutsch (Muttersprache)
Englisch (fließend)

EDV: MS Office

Hobbys/Interessen: Skifahren
Tanzen
Tauchen
Natur
Reisen
Literatur