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A dragonfly in the desert: genetic pathways of the widespread *Trithemis arteriosa* (Odonata: Libellulidae) suggest male-biased dispersal

Sandra Damm · Heike Hadrys

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Abstract Water-dependent species inhabiting desert regions seem to be a contradiction in terms. Nevertheless, many species have evolved survival strategies for arid conditions. In Odonates (dragonflies and damselflies), both larvae and adults require very different and complex water-associated habitat conditions. The present study investigates the genetic diversity, population structure and dispersal patterns of a desert inhabiting odonate species, the Red-veined Dropwing dragonfly, *Trithemis arteriosa*. Eight populations from the arid Namibia and four population sites in the more tropical Kenya were compared by using nine microsatellite loci, one non-coding nuclear fragment and the mtDNA fragment ND1. Microsatellite analyses as well as the nuclear fragment reveal a high allelic diversity in all populations with almost no genetic sub-structuring. In contrast, ND1 sequence analyses show sub-structuring and—with two exceptions—only private haplotypes. The conflicting patterns of nuclear versus mitochondrial markers suggest a male-biased dispersal in this species. Results indicate that male dispersal is dependent on the environmental stability of the habitat, while females are philopatric. This life history adaptation would allow females to save energy for mating and oviposition in the demanding environment of a desert region. The results give direct insights into the dispersal pathways of a desert-inhabiting, strongly water dependent flying insect.

Keywords Dragonflies · Desert regions · Microsatellites · mtDNA · Non-coding nuclear marker · Sex-biased dispersal

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S. Damm (✉) · H. Hadrys
ITZ, Ecology and Evolution, TiHo Hannover,
Bünteweg 17d,
30559 Hannover, Germany
e-mail: sandra.damm@ecolevol.de

H. Hadrys
Division of Invertebrate Zoology,
American Museum of Natural History,
New York, NY 10024, USA

Introduction

Dispersal is one of the key processes allowing the survival of species in fragmented landscapes and extreme environmental conditions. The "decision" to disperse can have far-reaching consequences for the fitness of individuals, e.g. founding of new populations (Clobert et al. 2001). Considering the potential benefits as well as the substantial risks associated with dispersal, it is highly plausible that dispersal might depend on actual environmental conditions (Bowler and Benton 2005; Gros et al. 2008). Analysing the population structure of key taxa in extreme environments could therefore help to understand dispersal strategies by taking into account the stability of habitat situations. Here, we analysed the genetic diversities and population structure of a desert-inhabiting dragonfly to gain first insights into the dispersal strategies of a water-associated insect in a desert environment.

Desert regions are one of the most challenging environments for living organisms. With no more than 100–500 mm precipitation per annum, water is the most limited resource in desert and semi-desert regions (Shmida 1985). Despite the extreme conditions, several species have evolved strategies for survival, e.g. adaptations for water conservation or heat tolerance (e.g. Ward 2009). However, studies of genetic diversity, population structures and dynamics for desert-inhabiting

species are limited, and have focussed mainly on mammals or other terrestrial organisms (e.g. Hurtado et al. 2004; Lorenzen et al. 2008; Sole et al. 2008). Even less is known about the genetic consequences of the limited availability of water bodies for freshwater-associated organisms inhabiting desert regions (Damm and Hadrys 2010).

Geographically, the study took place in Namibia, which is one of the most arid countries of the world. Most of the landscape is characterised by desert, semi-desert or dried savannah, with only three permanent rivers at the borders of the country (Mendelsohn et al. 2002). Other natural permanent water resources, like small springs and streams in mountainous regions, are rare and sometimes separated by large uninhabitable areas.

Odonates (dragonflies and damselflies) are highly dependent on water bodies, with their complex life cycle being composed of an aquatic larval and a terrestrial imago stage. They are highly mobile insects and the Anisoptera (dragonflies) in particular have the power to fly long distances. However, the dispersal potential of both dragonfly and damselfly species differs significantly in relation to specific habitat preferences (e.g. Corbet 1999; Damm et al. 2010a). While some are migratory species and are dispersed across whole continents (e.g. *Anax junius* or *Libellula quadrimaculata*), others (e.g. *Megaloprepus caerulatus* or *Trithemis hartwigi*) are dependent on highly specialised habitats (Artiss 2004; Dijkstra 2007; Fincke and Hadrys 2001; Freeland et al. 2003; Groeneveld et al. 2007).

In arid regions, some species groups have evolved real desert endemics like in reptiles or mammals (Griffin 1998; Simmons et al. 1998), but the majority of desert-inhabiting odonates are distributed widely across the African continent. They have evolved ecological strategies enabling them to survive under arid conditions (Johansson and Suhling 2004; Suhling et al. 2003). Most are more-or-less opportunistic in habitat preferences, and a short larval development enables some species to breed also in ephemeral water bodies during the rainy season (e.g. Suhling et al. 2005, 2009). In Namibia, 126 of an estimated 850 afrotropical odonate species have been identified, with the highest species diversity in the more humid and tropical parts of Namibia in the North (Dijkstra 2003; Suhling et al. 2006). Here, perennial and running waters allow more tropical species to inhabit the region. In the arid parts of Namibia, species diversity is poor and, in contrast to other animal groups, no endemic dragonfly species has been identified to date.

To study the genetic effects of dealing with rare water resources as water dependent species, the population structures of the Red-veined Dropwing *Trithemis arteriosa* (Burmeister 1839; Libellulidae) were analysed in different localities within Namibia and in the more tropical Kenya. The species distribution ranges from the semi-arid to tropical and humid regions across the African continent (Pinhey 1970). It is a habitat generalist and often a dominant species at perennial waters with emergent vegetation (Clausnitzer 2003). In Namibia, *T. arteriosa* was found only at permanent water ponds, mainly wetlands below lakes and spring brooks (Suhling et al. 2006). Here, population sizes at the examined localities differ widely depending on the stability of the habitat and water resource. As a consequence of the dry climate, Namibians freshwater systems are particularly threatened by both aridification and the impact of human activities (overuse of water, water pollution, extraction of groundwater for irrigation) (Barnard 1998). Such instability of water resources, together with the sometimes high geographical distances between permanent water ponds (especially in the South of Namibia), might influence the dispersal behaviour of the species.

In order to explore the population structures and genetic diversities of *T. arteriosa* in Namibia, three different genetic marker systems were used; microsatellites, mtDNA and a nuclear sequence marker.

Materials and methods

Study sites and sample collection

Samples of adult *T. arteriosa* individuals (n=129), representing 12 distinct geographical localities in Namibia and Kenya (see Table 1, Fig. 1), were collected and stored in 75 % ethanol. All sampled individuals are males, because females mostly stay away from the waterside and are often difficult to identify (e.g. Corbet 1999). Due to the species habitat preferences, all study sites are permanent water bodies, but abundances of *T. arteriosa* differ as a consequence of the type and quality of the habitats. At small populations, not more than 10 males were counted, at medium-sized populations 15-20 males, and at large population more than 20 males were counted. The most northern population site is located in the Baynes Mountains. Here, the species established a medium-sized population at a natural spring. The sites Palmwag and Ongongo are located in north-west Namibia. These populations were found at small ponds inside a dry riverbed, where *T. arteriosa* was able to establish larger populations. Waterberg is situated in the Northeast where a small *T. arteriosa* population was found at an artificial stream. The population site Rehoboth is located at the artificial lake Oanob, which provides water for the urban area around Rehoboth in South-central region of Namibia. Despite this rather atypical habitat, *T. arteriosa* established a medium-sized population. The population sites Tsauchab and Neuras are both located south of the great central Namibian escarpment. While Tsauchab is a permanent spring in a dry ephemeral river course with a

Table 1 Sampling locations with abbreviations and geographical coordinates as well as number of analysed individuals (*n*) of the Red-Veined Dropwing, *Trithemis arteriosa*, from Namibia and Kenya

Country	Abbreviation	Locality	Latitude	Longitude	*n*
Namibia	BayMt	Baynes Mountains	17.231 S	12.805 E	8
	Palm	Palmwag	19.887 S	13.937 E	19
	Ong	Ongongo	19.140 S	13.820 E	10
	Wb	Waterberg	20.483 S	17.235 E	9
	Reho	Rehoboth	23.301 S	17.031 E	11
	Neur	Neuras	24.463 S	16.228 E	11
	Tsau	Tsauchab	24.503 S	16.115 E	16
	FishR	Fishriver	24.498 S	17.863 E	9
Kenya	Pem	Pemba River	04.183 S	39.400 E	12
	Mzi	Mzima Springs	02.967 S	38.017 E	8
	NNP	Nairobi National Park	01.400 S	36.900 E	8
	LCh	Lake Chala	03.317 S	37.700 E	8

high abundance of *T. arteriosa*, the Neuras population is small, probably caused by human disturbance. The most southern site is a natural spring in the dry Fish River bed with a larger population (see Fig. 1).

For comparative analyses, four population sites in the more tropical region of Kenya were added to the study. Although Kenya possesses arid regions, it contains many more natural and permanent water resources than Namibia, for example the small natural Lake Chala in the South of Kenya. The other three population sites (Pemba River, Mzima Springs and Nairobi National Park) are permanent rivers and streams with riverine vegetation (Fig. 1). Here, *T. arteriosa* established medium-sized populations.

All samples were collected using a non-destructive method (Hadrys et al. 1993). The samples were stored at 4 °C in ≥70 % ethanol for consecutive DNA extraction. Extraction of total genomic DNA was carried out using a modified phenol-chloroform protocol (Hadrys et al. 1992) and stored at –20 °C.

Genetic analyses

For genetic analyses three different markers were chosen: the mitochondrial gene ND1 (NADH dehydrogenase subunit 1), which was successfully used in other genetic studies in odonates, as well as a non-coding nuclear fragment TartR04 (microsatellite flanking region), and a set of nine microsatellite loci (Damm et al. 2010b; Giere and Hadrys 2006).

A 610 bp fragment of ND1 was amplified and sequenced according to Rach et al. (2008). For amplification of a 301 bp fragment of TartR04 as well as the microsatellite loci, the primers and PCR regime described in Giere and Hadrys (2006) were used.

Purified PCR products were sequenced in both directions using an automated sequencer (MegaBACE500; Amersham Bioscience, Piscataway, NJ) and the ET Terminator Mix from Amersham Bioscience following the manufacturer's protocol. DNA sequences were assembled and edited using SeqmanII (version 5.03; DNAStar, Madison, WI). Consensus sequences were aligned using Clustal X version 1.8 (Thompson et al. 1997). To reconstruct the gametic phases in heterozygote individuals for the nuclear sequence marker, the Bayesian statistical method implemented in the program PHASE version 2.1 (Stephens et al. 2001) was used. Ten independent runs were conducted to infer the most confident haplotypes with a posterior probability greater than 95 % as suggested by the authors. Haplotype definition for ND1 and calculations of variable nucleotide positions were performed with Quickalign (Müller and Müller 2003). Sequences of each haplotype are available in GenBank under Accession nos FJ471463–FJ471481 (ND1) and JQ312300–JQ312328 (TartR04).

In addition, nine microsatellite loci described in Giere and Hadrys (2006) were used for genotyping. Amplified fragments were analysed on a MegaBACE500 (Amersham Bioscience) automated sequencer. Allele sizes were determined using the internal size standard ET-550 (Amersham Bioscience). Data analyses were performed using the Genetic Profiler software (version 1.2; Amersham Bioscience). MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to test for null alleles and allelic dropout using 1000 Monte Carlo simulations and a Bonferroni corrected 95 % confidence interval.

Statistical analyses

Genetic diversity

The genetic variation among mtDNA and nuclear sequences was quantified as haplotype diversity (*h*) and nucleotide diversity (π) and estimated using DNASP version 4.0 (Rozas et al. 2003). For the microsatellites, single locus statistics including number of alleles (*n*), allele frequencies (A/locus) and allelic richness (AR) were calculated using FSTAT version 2.9.3.2 (Goudet 2001). Observed (H_O) and expected (H_E)

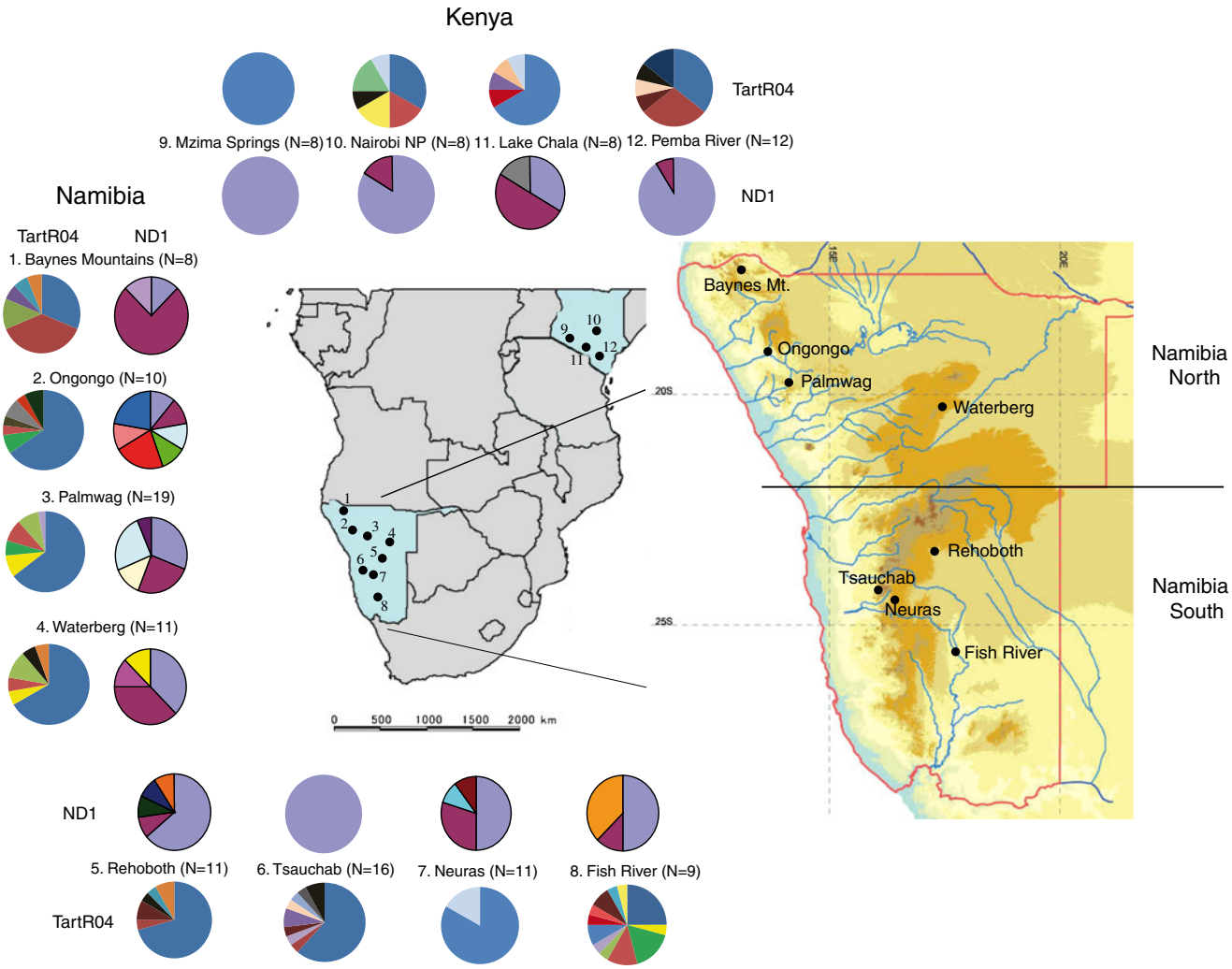

Fig. 1 Sampling localities in Namibia and Kenya (*left*) with a detailed map of Namibia (*right*) illustrating the ephemeral river catchments and the geological relief. *N* Numbers of individuals for each population. *Pie charts* display the haplotypes frequencies of ND1 and TartR04 found for each analysed population of *Trithemis arteriosa*

heterozygosities were calculated using GENEPOP version 4.0 (Rousset 2008). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested using the Markov chain method implemented in GENEPOP. Associated probability values were corrected for multiple comparisons using a Bonferroni adjustment for a significance level of 0.05 (Rice 1989). The entire dataset and the individual locality were tested for selective neutrality using Tajima`s *D* (Tajima 1989) and Fu`s *Fs* (Fu 1997) using ND1 and nuclear sequences. If Tajima`s *D* and Fu`s *Fs* are found to be significantly negative, the presence of selection or the occurrence of population growth is suggested.

Population structure

ARLEQUIN version 3.0 (Excoffier et al. 2005) was used for all markers to estimate genetic structuring between populations (F_{st}) and to conduct exact tests of population differentiation (Raymond and Rousset 1995). Hierarchical structuring of genetic variation was determined using analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented in ARLEQUIN. AMOVA estimates the amount of genetic variation attributable to genetic differentiation among predefined groups (Φ_{CT} and θ_{CT} for mtDNA and nuclear markers, respectively), among localities within groups (Φ_{SC} and θ_{SC}), and among localities relative to the total sample (Φ_{ST} and θ_{ST}). Analysing the distribution of variation five different groups of localities were compared as described in Table 3.

Statistical parsimony haplotypes networks were constructed for ND1 and TartR04 using the 95 % parsimony criterion as implemented in the TCS version 1.13 program (Clement et al. 2000; Templeton et al. 1992). Such genealogical networks provide a better representation of gene genealogies at the population level and also

allow relationships at the lower intraspecific level to be resolved.

For the microsatellites, the population structure was estimated using the model-based Bayesian approach implemented in STRUCTURE version 2.1 (Pritchard et al. 2000). Ignoring prior population notation, individuals were placed into *K* populations, which were genetic clusters with distinctive allele frequencies. Individuals were assigned probabilistically to populations, with membership coefficients summing to 1 across clusters. To provide the correct estimation of *K*, the ΔK statistic was used (Evanno et al. 2005). Runs with values of *K* from 1 to 12, corresponding to the numbers of sampled populations, were repeated 20 times. Using the admixture model with correlated frequencies, runs had a burn-in period of 10^5 steps followed by 10^6 Markov chain Monte Carlo replicates.

Mantel's test was performed to test for a correlation between geographic and genetic distance and as well as F_{st}-values using the program IBDWS version 2.6 (Jensen et al. 2005; Rousset 1997). Default settings were used, including 1,000 randomisations.

Results

Genetic diversity

ND1

Sequences of a 481-bp fragment of ND1 were obtained from all 129 individuals; 20 variable nucleotide positions were identified resulting in 19 different haplotypes. No deletions or insertions were observed. The two most common haplotypes (ART1 and ART2) were found in 69 % of all individuals. Haplotype ART1 occurred in all and haplotype ART2 in 10 (except Tsauchab and Mzima Springs) of the population sites analysed. The majority of haplotypes (16) were private and population specific (see Fig. 2a). An overview of haplotype frequencies per population is provided in Online Resource 1. Nucleotide sequence diversity (π) ranged from 0 to 0.99 % (Table 2). The populations Tsauchab and Mzima Springs exhibited only ART1 and therefore π and haplotype diversity (*h*) are zero (Table 2). The highest π was observed for Ongongo (0.99 %) followed by the populations of Waterberg (0.97 %) and Nairobi National Park (0.97 %). The highest *h* was again found in Ongongo (0.94) followed by Palmwag (0.81) and Waterberg (0.79). Both tests for selective neutrality (Fu`s *Fs* and Tajima`s *D*) were not significantly different from 0 in any analysed population, suggesting selective neutrality of the observed nucleotide polymorphism. Only population site Pemba has a significant negative *D* (–1.94, $P=0.009$), which might be caused by a recent population expansion.

TartR04

The 301 bp fragment of the nuclear microsatellite flanking region TartR04 showed 16 polymorphic sites and nine gaps. Two gaps are single deletions and the other seven gaps result from a 7-bp long insertion in five individuals occurring in different populations. Using the program PHASE 2.1 (Stephens et al. 2001), 29 haplotypes (including gaps and polymorphic sites) could be inferred with a posterior probability of 95 %. One haplotype (R04-1) occurred in all populations, followed by a second (R04-4) that is present in 9 out of the 12 populations (not in Mzima Springs, Neuras or Lake Chala). Thirteen haplotypes are shared by at least two populations, while 16 haplotypes are private (Fig. 2b). An overview of haplotype frequencies per population is provided in Online Resource 1. Nucleotide (π) and haplotype diversity (*h*) ranges from 0.1 to 0.89 % and 0.29 to 0.91, respectively, with the highest value of both π and *h* found for Nairobi National Park (0.89 %, 0.86) and Fish River (0.77 %; 0.91) (Table 2). Test for selective neutrality revealed significant negative *Fs* values for three populations, Tsauchab (–4.60, $P<0.001$), Waterberg (–2.51 $P=0.02$) and Lake Chala (–2.47, $P=0.006$).

Microsatellites

In total, 85 alleles were scored for the 12 analysed populations, and the number of alleles per locus ranged from 4 to 12. Allelic diversity ranged from 3.22 to 5.56 averaged over the nine loci. Allelic richness, which is based on the smallest sample size, ranged from 3.06 to 4.0 per population and locus (Table 2). The number of alleles found in populations ranged from 29 in Mzima to 50 in Palmwag. The highest number of private alleles was three and occurred in the Namibian populations Tsauchab, Neuras and Rehoboth as well as in the Kenyan population Pemba River. Observed heterozygosities across all loci ranged from 0.48 to 0.65 (Table 2).

Eight of the nine loci showed no evidence of null alleles. For the locus TartM04, the test for null alleles showed a significant value in six populations and deviations from the Hardy-Weinberg equilibrium in eight populations ($P<0.01$). Consequently this locus was excluded from further analyses. Furthermore, three populations (Lake Chala, Waterberg and Tsauchab) showed a significant deviation from Hardy-Weinberg equilibrium ($P<0.01$) indicating a heterozygote excess. For no combination of a pairs significant linkage disequilibrium was found.

Population genealogies

Two parsimony networks (ND1 and TartR04) illustrate the genealogical relationships between the haplotypes of all

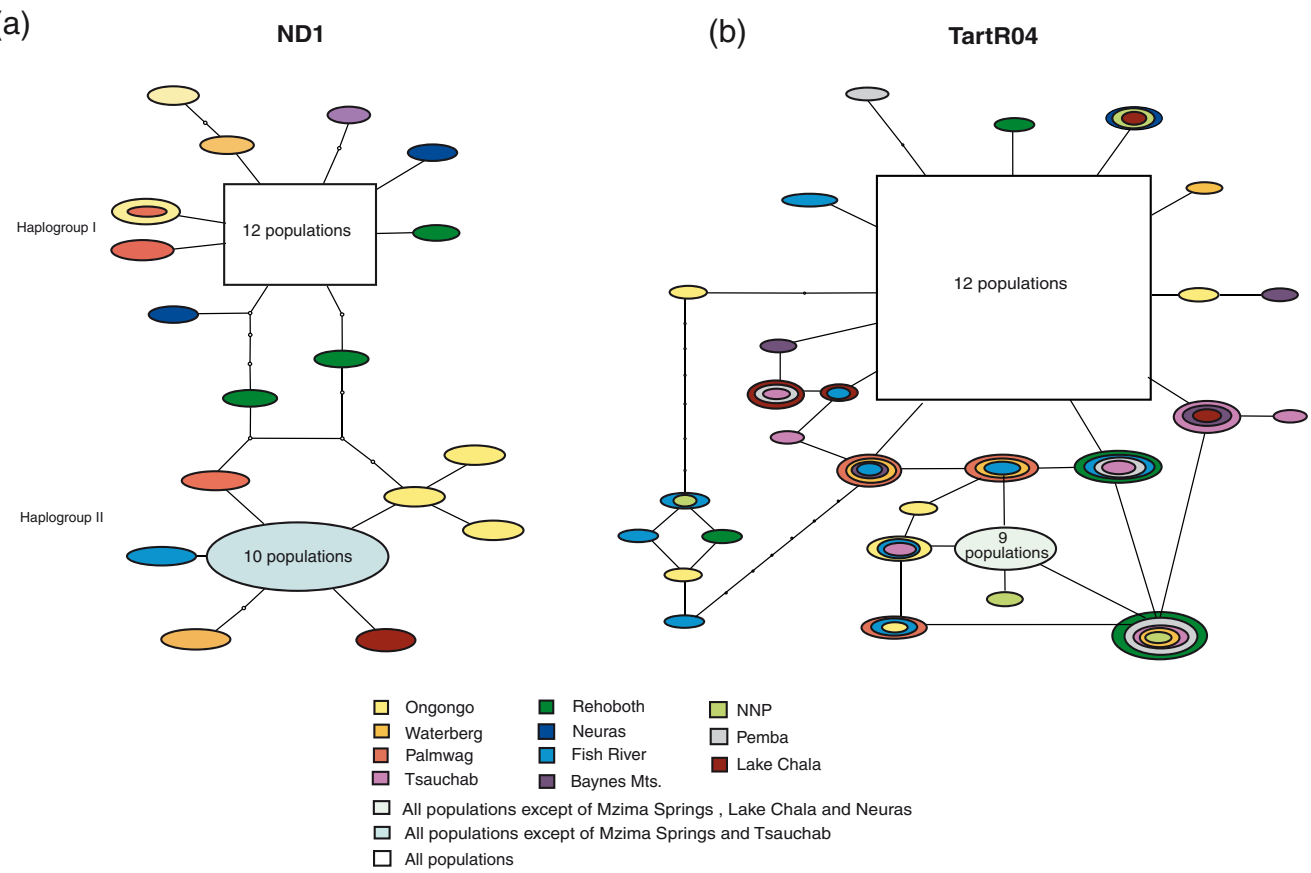

Fig. 2 Mutational haplotype networks of **a** ND1 and **b** TartR04 sequences based on statistical parsimony. Shown are the genealogical relationships between the haplotypes in 12 populations of *T. arteriosa.* Haplotypes considered to be ancestral are depicted as *rectangles*, all other haplotypes as *circles*. Missing mutational steps connecting haplotypes are represented by *small non-coloured dots*. Haplotypes connected by a *single line* differ in one mutational step. The size of the *rectangle* and *circles* correlates with haplotype frequency within each network. The different *colours* represent different populations

populations (Fig. 2a, b). For ND1, two haplogroups can be defined. Haplogroup I includes the most common haplotype ART1 in its central position and nine other haplotypes separated by only one to three mutational steps. Haplogroup II includes nine haplotypes separated by one to three mutational steps with haplotype ART2 in its central position. Interestingly, both haplogroups contain population sites from North and South Namibia as well as Kenya.

The haplotype network of TartR04 is dominated by one common haplotype (R04-1) occurring in all populations. Nineteen additional haplotypes are separated from R04-1 by only one or two mutational steps. A second haplotype (R04-4) separated by three mutations steps from the most common haplotype was found in 9 of the 12 populations and is connected with four further haplotypes separated by one to two mutation steps to R04-4. One group of five haplotypes is separated by at least eight mutations steps from the R04-1. This group contains individuals that have the 7-bp insert described above. Different from ND1 13 haplotypes of TartR04 are shared by at least two populations.

By means of AMOVA, a significant overall Φ_{ST}- and θ_{ST}-value was detected when comparing genetic variation among all populations for all three markers (ND1: 0.200**; TartR04: 0.07*** and microsatellites 0.03***) (Table 3). Hierarchical analysis of AMOVA revealed for all markers the highest variation within rather than among populations for all models tested (ND1: 75.12–80.85 %; TartR04: 92.93–93.57 %; microsatellites: 96.98–97.03 %). The variation among and within populations in the different defined groups as well as within the populations showed nearly no differences for TartR04 and the microsatellites. This resulted in the same level of significant θ_{SC}- and θ_{ST}-values (TartR04: 0.061 to 0.083***; microsatellites: 0.030 to 0.031***) while the θ_{CT}-values are not significant. In contrast, the variation in ND1 ranged among groups between –1.9 % and 12.38 % with Model 4 (Kenya and Namibia South / Namibia North) showing also a significant Φ_{CT}-value (0.123, P=0.001), which indicates a sub-structuring between these two groups. Here also, the Φ_{ST}-value was highest (0.248, P<0.0001). For the other models, the Φ_{ST}- value ranged from 0.191 to 0.213, indicating a substructure within the populations of each group.

Table 2 Mitochondrial DNA (ND1), nuclear sequence marker (TartR04) and nuclear microsatellite diversity in 12 *T. arteriosa* populations: Number of haplotypes; π nucleotide diversity; *h* haplotype diversity; *SD* standard deviation; *D* Tajima`s D; *Fs* Fu`s Fs; n number of alleles; *A/locus* number of alleles per locus; *AR* allelic richness corrected for sample size; H_o observed heterozygosities; H_e expected heterozygosities

	ND1					TartR04					Microsatellites				
Locality	Haplotypes (total/private)	$\pi \pm$ SD (%)	$h \pm$ SD	*D*	*Fs*	Haplotypes (total/private)	$\pi \pm$ SD (%)	$h \pm$ SD	*D*	*Fs*	*n* (total/private)	A/ locus	AR_c	H_o	H_e
BayMt	3 / 1	0.73±0.3	0.46±0.2	0.04	2.95	6 / 2	0.66±0.1	0.78±0.07			39 / 1	3.89	3.61	0.64	0.66
Palm	5 / 2	0.79±0.13	0.81±0.05	1.45	2.16	6 / 0	0.45±0.08	0.57±0.09	0.86	-0.67	50 / 1	5.56	3.50	0.5	0.59
Ong	7 / 4	0.99±0.21	0.94±0.07	0.38	-1.33	7 / 4	0.53±0.12	0.57±0.1	-0.46	0.11	38 / 2	4.22	3.44	0.49	0.61
Wb	4 / 2	0.97±0.1	0.79±0.11	1.70	2.03	6 / 1	0.33±0.1	0.56±0.13	-0.47	-2.51*	39 / 2	4.33	3.56	0.48*	0.60
Reho	5 / 3	0.47±0.18	0.62±0.16	-0.66	-0.07	6 / 2	0.36±0.1	0.50±0.12	-0.99	0.31	46 / 3	5.11	4.00	0.65	0.74
Neur	4 / 2	0.78±0.17	0.71±0.12	0.75	2.12	2 / 0	0.1±0.04	0.29±0.12	0.02	0.46	40 / 3	4.44	3.54	0.62	0.62
Tsau	1 / 0	0	0	0	0	9 / 2	0.43±0.09	0.62±0.11	-0.96	-4.60*	46 / 3	5.11	3.77	0.55*	0.65
FishR	3 / 1	0.94±0.15	0.68±0.12	2.27	3.71	12 / 3	0.77±0.07	0.91±0.04	0.16	-2.44	35 / 0	3.89	3.23	0.52	0.61
Pem	2 / 0	0.24±0.19	0.17±0.13	-1.94*	2.76	6 / 1	0.60±0.1	0.81±0.10	0.38	-0.62	48 / 3	5.33	3.84	0.55	0.65
Mzi	1 / 0	0	0	0	0	1 / 0	0	0	0	0	29 / 1	3.22	3.22	0.56	0.55
NNP	2 / 0	0.97±0.45	0.67±0.31	0	2.88	6 / 1	0.89±0.1	0.86±0.07	0.51	0.87	35 / 1	3.89	3.06	0.48	0.67
LCh	3 / 1	0.84±0.24	0.73±0.15	0.94	2.47	5 / 0	0.30±0.1	0.58±0.16	-1.38	-2.47*	39 / 1	4.33	3.69	0.58*	0.66

*Significant values

Pairwise Φ comparisons (ND1) varied widely. Of the 66 population comparisons, 20 showed significant Φ_{ST}-values after Bonferroni correction (values ranging from 0.012 to 0.758). While some of the high values might have been caused by low nucleotide diversities (Tsauchab and Mzima Springs), the main significant Φ_{ST}-values were found between northern Namibian populations and Kenya. Pairwise θ_{ST} comparisons for TartR04 showed 25 significant pairwise comparisons out of 66 (values ranging from 0.075 to 0.364). Here, two populations (Fish River and Baynes Mountains) showed the majority of the significant θ_{ST}-values compared to nearly all other populations. For the microsatellites, pairwise θ_{ST} comparisons showed 27 significant θ_{ST}-values, which were slightly higher than in the AMOVA analyses (ranging from 0.019 to 0.103). The highest θ_{ST}-value was found between Fish River and Lake Chala with 0.103 ($P<0.0001$). The most significant values were found between northern and southern populations of Namibia, and again between Fish River and the other populations. Pairwise population F_{st}-values for all three markers are provided in Online Resource 1.

According to the substructuring between northern to southern Namibian and Kenyan populations in Φ_{ST} and θ_{ST}, the exact test of population differentiation was performed (1) for population comparison, and (2) for groups of populations (North Namibia vs South Nambia vs Kenya). Microsatellites revealed no significant differentiation for both population and group comparison. ND1 and TartR04 showed the same pattern of population pairwise differentiation as revealed by Φ_{ST}- and θ_{ST}-values. Testing the differentiation of the three predefined groups, both markers showed a significant differentiation between North Namibia and South Namibia as well as Kenya while the latter two were not differentiated significantly from each other.

The model-based clustering method implemented in STRUCTURE (Pritchard et al. 2000), which assigns all individuals to *K* clusters without predefined populations, was performed for (1) all populations separately, and (2) five predefined groups according to exact population differentiation results of ND1 and TartR04 (North Namibia, South Namibia, Kenya and the two highly differentiated populations Fish River and Baynes Mountains). This was done to allow higher sample sizes for each geographical region. For both approaches, $K=3$ produced the highest value of ΔK. Nevertheless, a high degree of overlap among individuals from different populations and regions was found, indicating high gene flow between the populations (Fig. 3).

Mantel tests for the three markers showed no significant correlation between geographic and genetic distances (ND1: r=–0.0897, one-sided p=0.7410; TartR04: $r=0.0470$, one-sided $P=0.6510$; microsatellites: Nei`s distances: $r=0.1274$,

Table 3 Distribution of genetic variance via hierarchical AMOVA. For nuclear and mitochondrial markers (ND1, TartR04 and microsatellites) five different groupings were tested. Kenya (represented by the populations Pem, Mzi, NNP, LCh), Namibia North (BayMt, Palm, Ong, Wb) and Namibia South (Reho, Neur, Tsau, FishR). For abbreviations, see Table 1

	ND1		TartR04		Microsatellites	
Source of variation	Variation	Fixation	Variation	Fixation	Variation	Fixation
Model 1 (without grouping)						
Among populations	20.03 %		7.07 %		3.02 %	
Within populations	79.97 %	θ_{ST}=0.200***	92.93 %	θ_{ST}=0.07***	96.98 %	θ_{ST}=0.030***
Model 2 (Namibia) (Kenya)						
Among groups	-1.9 %	θ_{CT}=-0.018	-1.71 %	θ_{CT}=-0.017	-0.02 %	θ_{CT}=-0.000
Among populations within groups	21.05 %	θ_{SC}=0.205**	7.76 %	θ_{SC}=0.076***	3.03 %	θ_{SC}=0.030***
Within populations	80.85 %	θ_{ST}=0.191**	93.95 %	θ_{ST}=0.061***	96.99 %	θ_{ST}=0.030***
Model 3 (Namibia North) (Namibia South) (Kenya)						
Among groups	5.87 %	θ_{CT}=0.058	-1.88 %	θ_{CT}=-0.019	-0.09 %	θ_{CT}=-0.001
Among populations within groups	15.44 %	θ_{SC}=0.154	8.44 %	θ_{SC}=0.083***	3.08 %	θ_{SC}=0.031***
Within populations	78.58 %	θ_{ST}=0.213	93.44 %	θ_{ST}=0.066***	97.01 %	θ_{ST}=0.030***
Model 4 (Namibia South, Kenya) (Namibia North)						
Among groups	12.38 %	θ_{CT}=0.123*	-0.77 %	θ_{CT}=-0.008	-0.1 %	θ_{CT}=-0.001
Among populations within groups	12.5 %	θ_{SC}=0.142***	7.51 %	θ_{SC}=0.074***	3.07 %	θ_{SC}=0.031***
Within populations	75.12 %	θ_{ST}=0.248***	93.27 %	θ_{ST}=0.067***	97.03 %	θ_{ST}=0.030***
Model 5 (Namibia North, Kenya) (Namibia South)						
Among groups	0.62 %	θ_{CT}=0.006	-1.46 %	θ_{CT}=-0.014	-0.05	θ_{CT}=-0.001
Among populations within groups	19.66 %	θ_{SC}=0.198***	7.89 %	θ_{SC}=0.078***	3.04	θ_{SC}=0.030***
Within populations	79.71 %	θ_{ST}=0.203**	93.57 %	θ_{ST}=0.064***	97.01	θ_{ST}=0.030***

* P<0.05, ** P<0.001, *** P<0.0001

P=0.1880; θ_{ST}: r=0.0142, P=0.4440), suggesting that no significant isolation by distance can be found.

Discussion

The application of two nuclear and one mitochondrial sequence markers revealed contrasting patterns in the population genealogies of *T. arteriosa*. The two nuclear markers showed highly similar results, indicating no population substructuring and high levels of gene flow between populations. In contrast, the mtDNA marker ND1 showed nearly exclusively private haplotypes in each population, suggesting reduced gene flow between populations.

Genetic diversity

With up to 7 (ND1) and 12 (TartR04) haplotypes and 50 alleles, high nucleotide and haplotype diversities were found in nearly all populations. Interestingly, in ND1 the highest diversities were found in the northern Namibian populations (Palmwag, Ongongo and Waterberg), while in the microsatellites and TartR04 no pronounced difference could be observed. The number of ND1 haplotypes was lowest in the Kenyan populations. The high overall genetic diversities in the northern part of Namibia lead to the assumption that these populations have been stable and in HWE over a long time period. In contrast, the southern populations of Namibia show lower mtDNA diversities, but a high number of private alleles and high haplotype diversity in TartR04. This suggests a past population decrease as a cause of more instable habitat conditions.

The Tsauchab population in South Namibia exhibits only one mtDNA haplotype although the number of individuals analysed was high. In contrast, a high number of microsatellite alleles (n=46) and nuclear haplotypes (nine including two private) were found. The loss of mtDNA diversity might be a response to climatic fluctuations resulting in a desiccation of water resources and a repeated decline in population size at this site. This is also supported by a significant Fu`s *Fs* value (–4.60, P<0.001) in TartR04 indicating a recent bottleneck or population expansion (Fu 1997; Tajima 1989).

The population Mzima in Kenya exhibited low genetic diversity in all three markers, which might have been caused by a recent population decline. The two populations Fish River (most southern) and Baynes Mountains (most northern) have a high TartR04 haplotype diversity and show different haplotype frequencies (ND1 and TartR04) in comparison to the other populations. This might be caused

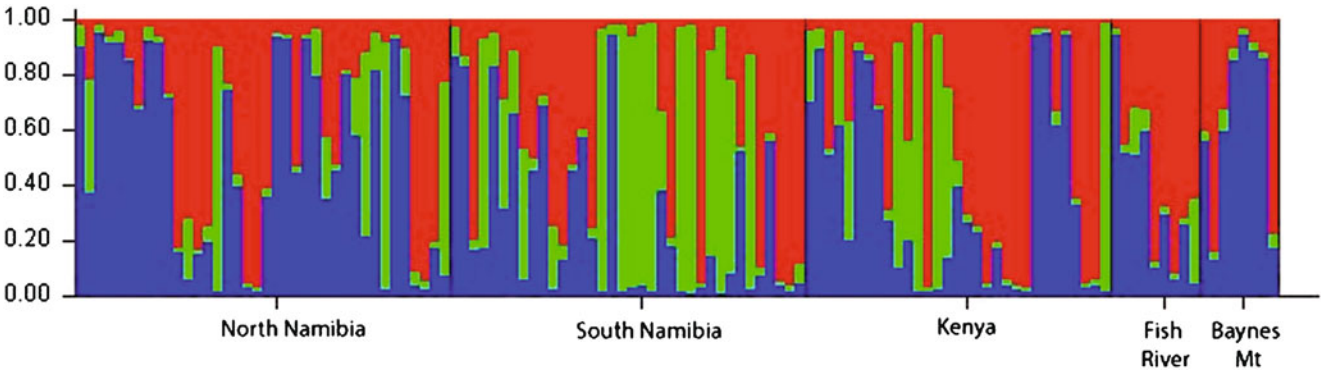

Fig. 3 Bayesian analysis of the nuclear genetic structure of *T. arteriosa* populations based on eight microsatellite loci. Each *vertical bar* represents an individual and is partitioned into one to three coloured segments indicating the individual membership in the three genetic groups found by STRUCTURE. Regional origin of the individuals is indicated by regarding the two populations Fish River and Baynes Mountains separately according to their high θ_{ST}-values compared to the other populations

through additional genetic input from populations of the adjacent countries South Africa and Angola.

When comparing the genetic diversity patterns of *T. arteriosa* in the dry country Namibia with the more tropical Kenya, lower diversities in the more demanding and isolated habitats of Namibia are expected. Interestingly, our study revealed rather the opposite. The Namibian sites showed a higher overall genetic diversity, with higher number of private haplotypes and alleles in all three markers. The Kenyan populations exhibit only the two most common haplotypes in ND1 and mostly shared haplotypes in TartR04 (with the southern Namibian populations). Several explanations are possible. Both Lake Chala (in TartR04) and Pemba (in ND1) showed a significant negative Tajima *D* or Fu`s *Fs*. Common population decline may result in the loss of genetic diversity favouring only the most common haplotypes. The more stable habitats in Kenya may harbour a higher number of predators (e.g. fish, frogs), interspecific competition, mammals or anthropogenic habitat disturbance. In Namibia the two populations Neuras and Waterberg with the most anthropogenic influence are indeed small in size and show low genetic diversity.

Population differentiation

The results of population structure analyses revealed different patterns when comparing mtDNA and nuclear markers. The nuclear markers showed nearly no population substructure, suggesting a high level of gene flow between the analysed populations. The TCS-network of TartR04 and the STRUCTURE analyses in the microsatellites showed no geographical correlation of haplotypes or allele frequencies and nearly half of the TartR04 haplotypes are shared by at least two populations (see Fig. 2b). In contrast, ND1 exhibited only private haplotypes (except for the two main haplotypes), and a population substructure between North and South Namibia was found (see Table 3).

An explanation for the substructure between North and South Namibia might be found in the geographical relief of Namibia. While all southern and central populations are situated on the southern escarpment and the Khomas highlands, the northern populations are located below these plateaus. The reduction in gene flow might therefore be caused by the gap of the escarpment and, along with that, a lack of suitable habitats in between (Suhling et al. 2009). The Naukluft Mountains are situated within the southern escarpment (location of the populations Tsauchab and Neuras) as well as the populations Rehoboth and Fish River. Part of the great canyon of the ephemeral Fish River has, in contrast to the other river catchments, a north–south direction and originates at the southern Namibian border at Orange River. Migration might therefore be southwards along the Fish River Canyon in the direction of South Africa (see Fig. 1). While the northern population sites have more constant climatic conditions, the southern Namibian populations are more affected by drought through the periodic absence of rain in the rainy season (Mendelsohn et al. 2002).

Interestingly, the Kenyan populations are related more closely to the southern Namibian populations than to the northern populations, as supported by both ND1 and TartR04. The migration of *T. arteriosa* from Kenya to Namibia might follow the coastline of southern Africa with the coastal wind and enter Namibia from South Africa. To prove this hypothesis, genetic analyses of populations between Kenya and Namibia as well as South Africa will be required, but genetic analyses of other species (*Orthetrum chrysostigma* and *Orthetrum julia)*, reveal the same picture (Damm and Hadrys 2010).

Contrasting patterns via sex-biased dispersal?

Although comparisons of mtDNA, nuclear sequence markers and microsatellites are complicated because of their different characteristics (allelic variation at specific loci vs

mtDNA sequence variation), similar patterns of genetic differentiation are expected if gender-based dispersal can be excluded (Bos et al. 2008; Lukoschek et al. 2008). In *T. arteriosa*, mtDNA analyses revealed, with the exception of two main haplotypes, only private haplotypes in each locality. In contrast, microsatellites and nuclear DNA analyses revealed mostly shared haplotypes with no indication of genetic differentiation. Microsatellite analyses require high sample sizes to assure that the genetic diversity of a population is covered (e.g. Waples 1998). We are aware that this requirement is not covered when comparing single sites. Therefore we included a non-coding nuclear sequence marker (TartR04), which mirrored and confirmed microsatellite results.

One obvious reason for the contrasting genetic population structure of mt versus nuclear DNA could be the fourfold-reduced effective population size of the maternal inherited mtDNA in comparison to the diploid/ bi-parentally inherited nuclear markers (Birky et al. 1989). Thus, theoretically, mtDNA may show higher levels of differentiation at a mutation-drift equilibrium compared to microsatellites, although mutation rates for microsatellites are higher. But the high number of mtDNA haplotypes in *T. arteriosa* in general and the high level of gene flow revealed by nuclear markers would suggest that at least some of these haplotypes are shared with other populations.

A second, highly promising explanation for the incongruence of mtDNA and nuclear data is sex-biased dispersal. Male-biased dispersal could homogenize allele frequencies among populations at biparentally (nuclear), but not maternally (mitochondrial) inherited genetic markers (e.g. Prugnolle and de Meeus 2002). Consequently, sex-specific dispersal can lead to incongruent results of analyses on population structures when comparing nuclear with mitochondrial markers. Male-biased dispersal is well studied in different vertebrate species, including mammals (e.g. Mesa et al. 2000), birds (e.g. Dallimer et al. 2002; Gibbs et al. 2000), and fishes (e.g. Cano et al. 2008).

In dragonflies, it is well known that, in the majority of species, females stay away from the waterside and arrive only for mating and oviposition, while male dragonflies compete for mating opportunities at the water (e.g. Corbet 1999). Competition in large populations with spatial limitations leads to evasion to new water resources and therefore dispersal (Perrin and Mazalov 2000). Also the costs for dispersal might differ between genders, resulting in the dispersal of only one sex (Gros et al. 2008). For females, staying at breeding sites and saving energy for mating and oviposition is of special importance when one regards the uncertainty of reachable appropriate habitats (in *T. arteriosa* permanent water ponds) in an arid region such as Namibia. Such a mating system, where males disperse to search for new territories, and mates and females are philopatric, has many advantages under challenging habitat conditions. Sex-biased dispersal might therefore be a life cycle adaptation to arid conditions. This is also described well in some studies of desert-inhabiting fruit flies (Markow and Castrezana 2000).

To prove this hypothesis, genetic analyses of females could give additional information, and by using a capture-mark-recapture (CMR) method the sex-ratio at the given population sites could be measured. Studies analysing the sex-ratio of some odonate species showed a slightly higher numerical predominance of males as well as a lower survival rate of females in several odonates species (Cordero Rivera and Stoks 2008; Kery and Juillerat 2004). Such different sex-ratios could also result in lower genetic diversities of the mtDNA, but might presumably not cause the degree of difference between mt and nuclear DNA found here. However, without estimation of sex-ratios, a combination of the two aspects (higher mortality and lower dispersal) cannot be ruled out.

Because of their high mobility, dispersal patterns in dragonflies are difficult, and without genetic information often impossible, to assess (Holland et al. 2006). Some past studies have analysed the dispersal propensity of different odonate species using CMR (Angelibert and Giani 2003; Beirinckx et al. 2006; McCauley 2005). In some European damselflies species, a female-biased dispersal was found (Beirinckx et al. 2006), which could be explained by, amongst other factors, the different foraging behaviour of the sexes. In general, CMR studies are often limited, especially in dragonfly species (e.g. high dispersal potential, difficult to catch). In the only study on a libellulid species, the males of *Leucorrhina intacta* show a difference in dispersal rates dependent on habitat conditions (McCauley 2005).

Based on our genetic results, dispersal rates of males in *T. arteriosa* also seem to be correlated with the environmental conditions of the habitat at the specific localities. While smaller population sites (Neuras, Waterberg, Mzima Springs) exhibit a lower genetic diversity and share most of their nuclear haplotypes with other populations, the populations with a long-term stable history have a higher genetic diversity and a higher number of private nuclear haplotypes and alleles (Ongongo, Fish River, Baynes Mountains, Rehoboth).

Therefore, a decrease in food and/or mating resources might have led to dispersal, which in fact could be a male-biased facultative dispersal. This picture is best mirrored by the genetic results of the Tsauchab population, where only the most common ND1 haplotype was found. Located in one of the driest parts of Namibia, recurrent drying events of the water resource might have led to a nearly complete migration of the males. While females do not migrate and stay at their breeding sites, the maternal inherited haplotypes in ND1 stay private for the specific locality and, in founder

events, this resulted in the occurrence of a low number of maternal lineages, as shown for the Tsauchab population.

Conclusions

A major problem in population genetic studies is the availability of individuals sampled at one site to correctly evaluate population structures. This is especially true for endangered species or species inhabiting extreme environments where sometimes only small and/or isolated populations can be established. The application of mtDNA and microsatellites in population genetic studies has proved a powerful technique (e.g. Avise et al. 1987; Goldstein and Schlötterer 1999). However, especially microsatellite analyses are highly dependent on the number of analysed individuals of a given population. In our study, the use of a third marker system, a non-coding nuclear sequence marker, resulted in congruent patterns when compared to microsatellites. In this way we were able to verify the microsatellite results (Zhang and Hewitt 2003). A microsatellite flanking region might therefore be a promising marker for population genetic studies. Although the number of analysed individuals in some populations is low (which correlates with species abundance), the observed patterns in genetic diversity could be correlated with the stability of water resources rather than with sample size (Tsauchab, Pemba River, Ongongo). Even when combining populations within the same geographical region, the overall picture did not change. This is additional evidence that using a non-coding nuclear region as a complement to mtDNA or/and microsatellites might allow population genetic structures to be reconstructed even in smaller sample sizes.

By applying the three different marker systems we could show that a desert-inhabiting species dependent on perennial waters is able to establish viable populations with high genetic diversities despite their isolated situation. In the desert, dispersal ability is of high importance as populations are always at risk of a spatial or total desiccation of water resources either by human impact or natural causes. While some species are obligatory migrants, others may disperse for foraging, reproduction or seasonally induced reasons. In *T. arteriosa*, genetic analyses suggest a male-biased dispersal that seems to be dependent on the stability of the habitat. While for females philopatry seems to be a fitness-advantage, males are forced to migrate in times of drought or habitat disturbance to search for other suitable habitats.

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