ORIGINAL ARTICLE

Phylogeography, pre-zygotic isolation and taxonomic status in the endemic Cyprus Wheatear *Oenanthe cypriaca*

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Abstract The insular endemic Cyprus Wheatear *Oenanthe cypriaca* has been considered as a subspecies of Pied Wheatear *O. pleschanka*. However, due to several differences in behaviour, habitat selection and morphology, it is currently treated by most authors as an independent species. Here, we used mitochondrial nucleotide sequences of the cytochrome oxidase subunit 1 gene (679 base pairs), playback experiments and dummy presentations to assess the status of *O. cypriaca*. For the playback experiments we used the conspecific song, and heterospecific songs of the two subspecies of Black-eared Wheatear *O. hispanica hispanica* and *O. hispanica melanoleuca*, *O. pleschanka*, and Finsch's Wheatear *O. finschii*. Experimental dummy presentations included *O. cypriaca*, *O. pleschanka* and a dark and light morph of *O. h. melanoleuca*. *O. cypriaca* responded significantly stronger towards the conspecific model and towards conspecific playbacks than towards heterospecific stimuli. ML and BI analyses support the close relationship between *O. cypriaca*, *O. pleschanka* and *O. h. melanoleuca*. With a relative high posterior probability value (0.98), *O. cypriaca* clusters closer to *O. h. melanoleuca* from Iran and Israel (on migration) and *O. pleschanka* from Iran than to *O. pleschanka* obtained from Kazakhstan, Russia, Mongolia and wintering areas in East Africa (Kenya). The scenario suggests that *O. cypriaca* might be either a relatively young taxon, which is yet behavioural distinct, but genetically still similar to its sister populations on the mainland. Alternatively, we may assume a close relationship as an indication for potential ongoing hybridisation processes involving all three forms.

Zusammenfassung

Phylogeographie, Taxonomie und präzygote Isolation beim Zypernsteinschmätzer *Oenanthe cypriaca*

Endemismus auf Inseln ist ein bekanntes Phänomen. Der Zypernsteinschmätzer *Oenanthe cypriaca* brütet nur auf Zypern und wurde lange Zeit als Unterart des Nonnensteinschmätzers *O. pleschanka* bezeichnet, obwohl einige Autoren besonders aufgrund des Gesangs ihn als eigene Art betrachteten. In dieser Studie untersuchten wir die mitochondrielle Nukleotidensequenz der Cytochrome oxidase subunit 1 um das phylogeographische Muster und die genetische Struktur von *O. cypriaca* zu bestimmen. Zusätzlich wurden noch Playbackexperimente und Modellpräsentationen durchgeführt (dummies). Dies sollte einen Hinweis auf die präzygote Isolation geben. Die Studie fand im März/April

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2008 und Mai 2009 statt. Verschiedene Playbacks wurden benutzt: konspezifischer Gesang und heterospezifischer von Mittelmeersteinschmätzer *O. h. hispanica/melanoleuca*, *O. pleschanka* und Felsensteinschmätzer *O. finschii*. Experimentelle Dummy Präsentationen beinhalteten Modelle von *O. cypriaca*, *O. pleschanka* und sowohl eine dunkle als auch eine helle Morphe von *O. h. melanoleuca*. *O. cypriaca* reagierte signifikant stärker auf konspezifischen Gesang und konspezifische Modelle als auf *O. pleschanka* und die anderen *Oenanthe*-Arten. 679 Basen Paare (bp) der mitochondriellen Cytochrome *c* oxidase subunit 1 (*CO1*) wurden untersucht. Die Maximum-Likelihood und Bayesian Inference Analysen unterstützten die nahe Verwandtschaft zwischen *Oenanthe cypriaca*, *O. pleschanka* und *O. h. melanoleuca*. Dieselbe Analyse allerdings zeigte interessanterweise, dass—mit hoher bootstrap Wahrscheinlichkeit—*O. cypriaca* ein Cluster bildet, indem die Art näher mit *O. h. melanoleuca* aus Iran und Israel (vom Durchzug) und *O. pleschanka* aus Iran als mit *O. pleschanka* aus Überwinterungsgebieten in West Afrika (Kenia) verwandt ist. Die Daten dieser Studie unterstützen teilweise den Status des Zypernsteinschmätzers als eigenständige Art, aber die molekulare Analyse zeigt, dass die Artbildung wahrscheinlich noch sehr jung ist oder Hybridisationsprozesse stattfinden.

Introduction

Geographic isolation—especially on islands—is one main factor of intraspecific differentiation, speciation and endemism (Price 2008), and it is interesting to see the historical change in how island forms are being treated by taxonomists. Under the broad biological species concept, many island forms have been treated as subspecies of widespread species of the adjacent mainland, under the assumption that they would probably hybridise if they were to meet. In more recent times, the trend is rather to recognize clearly diagnosable island forms as separate evolutionary units, and to give them status as distinct species. On a European scale, island endemism and evolution of discrete species is reported from Macaronesia (see e.g. Päckert and Martens 2004; Dietzen et al. 2003, 2005, 2008a, b; Gonzalez et al. 2009) and from the Mediterranean (e.g. Brambilla et al. 2008; Förschler et al. 2009).

On Cyprus, the Cyprus Wheatear *Oenanthe cypriaca* has been considered as distinct species by different authors because of morphometric measurements, less pronounced sexual dichromatism and significantly different song than the Pied Wheatear *O. pleschanka* (Homeyer 1884; Christensen 1974; Sluys and van den Berg 1982; Bergmann 1983; Flint and Stewart 1992; Flint 1995; Förschler et al. 2010), while other authors retained it as a subspecies of *O. pleschanka* (e.g. Panov 2005). Behavioural observations

and ecological studies by Randler and Crabtree (2010) provided additional evidence for the splitting, and in a recent work it has been shown that both forms are very well separated by several morphological traits (Förschler et al. 2010). However, molecular studies have not been carried out to support the morphological, behavioural and bioacoustic evidence. Above and beyond the molecular analysis, prezygotic isolation—a key factor of the biological species concept—should also provide further evidence for species isolation (e.g., in assortative mating; Randler 2008). As there are no overlapping breeding areas with other *Oenanthe* species, hybridisation—which is common in *Oenanthe* species (Cramp 1988; Panov 2005; Randler 2006; Aliabadian et al. 2007)—could not be assessed as a pre-requisite for the biological species concept (e.g. Randler 2002, 2004), and we therefore used playback experiments and model (dummy) presentation to simulate the intruding of heterospecifics. In contrast to the Macaronesian and many tropical island species, the Cyprus Wheatear is a migratory species, and thus meets congeners during migration and on the winter quarters, and this makes a thorough study of potential gene flow and reproductive isolation highly interesting.

In this study, using mitochondrial nucleotide sequences of the cytochrome oxidase subunit 1 gene, we aim to analyse the phylogeographical and genetic structure of the Cyprus Wheatear compared to closely related taxa such as the Pied Wheatear and the two recognised subspecies of Black-eared Wheatear (*O. h. hispanica* and *O. h. melanoleuca*). By using playbacks and dummy presentations, we further assess the pre-zygotic isolation in *O. cypriaca*. The strength of the present study is the combination of molecular data with aspects of pre-zygotic isolation.

Methods

Study area

Cyprus is an island in the south-eastern Mediterranean, approximately 225 km long and 100 km wide. It is the third largest island in the Mediterranean (9,250 km^2) after Sicily and Sardinia (Stagg and Hearl 1997), and lies less than 100 km from Turkey, and less than 200 km from Syria and Lebanon at 34°33′–35°42′N, 32°16′–34°36′E (Jones 2006). The island is dominated by two mountain ranges, the Troodos mountains in the south rising to 1,961 m and further north, the Kyrenia range rising to 1,024 m. Cyprus has an extreme Mediterranean climate with long, very hot, dry summers and cool, wet, changeable winters (Flint and Stewart 1992). The island has a variety of natural vegetation; 18% of the island is woodland (Stagg and Hearl 1997; Förschler and Randler 2009).

DNA isolation and sequencing of the mitochondrial *CO1* gene

Samples from *Oenanthe* species were genetically analysed from different populations located in Cyprus, Israel, Asia and Africa (for details, see Table 1, and Fig. 2, below). The DNA was obtained from blood or growing feathers. Total DNA was isolated using standard proteinase K (Merck, Darmstadt) and phenol/chloroform procedures (Sambrook et al. 1989).

We amplified a fragment of the mitochondrial cyto-chrome *c* oxidase subunit 1 (*CO1*) gene using the primers *passerF1* and *passerR1* (Lohman et al. 2009). The PCR amplifications were performed in 50-µl reaction volumes containing 1× PCR buffer (Bioron, Ludwigshafen), 100 µM dNTPs, 0.2 units of *Taq* DNA polymerase (Bio-ron), 200 ng of DNA and 5 pmol of primers. Optimal annealing temperature was found by gradient PCR in a Tgradient thermocycler (Biometra). Thermal cycling was performed under the following conditions: 5 min at 94°C, followed by 35 cycles of 40 s at 94°C, 40 s at 52.0°C, 1 min at 72°C and a final extension at 72°C for 10 min. PCR products were precipitated with 4 M NH_4Ac and ethanol (1:1:6) and centrifuged for 15 min (13,000 rpm).

Sequencing was performed using a ABI 3730 automated capillary sequencer (Applied Biosystems) with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 by STARSEQ (Mainz, Germany). In order to confirm observed mutations, both strands of each sample were sequenced. All sequences generated in this study have been deposited in GenBank under accession numbers HM126495–HM126527.

The nucleotide sequences were aligned manually with BioEdit version 7.0.9.0 (Hall 2004). No internal stop codons or frame-shifts were observed in the sequences that were translated entirely by using the chicken mitochondrial code. The typical mitochondrial pattern of predominantly third codon position substitutions was evident (Moore and DeFi-lippis 1997). Basic statistics and average uncorrected *p* distances were calculated with MEGA version 4.0 (Tamura et al. 2007). Phylogenetic trees were reconstructed using maximum likelihood (ML) in PAUP* version 4.0b10a (Swofford 2002) and Bayesian inference (BI) in MRBAYES version 3.1.2. (Ronquist and Huelsenbeck 2003). Following Outlaw et al. (2010), the trees were rooted using several species of stonechats (*Saxicola*), rock-thrushes (*Monticola*), the Orange-flanked Bush-robin (*Tarsiger cyanurus*), two species of nightingales (*Luscinia luscinia* and *L. megar-hynchos*) and the Rufous-backed Redstart (*Phoenicurus erythronotus*) as outgroups (see Table 1).

We explored the model of sequence evolution that fits the data best with MODELTEST version 3.7 (Posada and Crandall 1998) and MRMODELTEST version 2.3 (Nylander 2004). ML heuristic searches were performed

with closest stepwise sequence additions, tree-bisection-reconnection branch-swapping (TBR), 'multrees' option and the best model found with MODELTEST. In the ML analyses, the robustness of each node was assessed by 1,000 bootstrap replicates. For BI analyses, two indepen-dent runs of 8,000,000 generations each were performed along with four Markov chains. Trees were sampled every 500 generations and the first 4,000 samples were discarded as 'burn-in'. The evolutionary models selected for BI and ML analyses were GTR + G + I and HKY + I + G, respectively.

Dummy presentation

Dummies were presented in eight different territories of *O. cypriaca*. The dummies were identical in size and struc-ture, but differed in their coloration (producer: artfauna). The four male dummies represented three species (Fig. 1), *O. cypriaca*, *O. pleschanka*, and *O. h. melanoleuca*. The latter species was presented in two distinct morphs, the light-throated and the dark throated morph. *O. pleschanka* is closest in coloration to *O. cypriaca*, and the light-throated morph of *O. h. melanoleuca* differs most. Dummy presen-tations were carried out between 11 and 18 May 2009, when weather conditions were good (no rain, less wind), between 0700 and 1300 hours local time. The dummy was placed always at the same post (e.g. a large stone) where it was widely visible to the territory owner. In each of the eight territories, all four models were presented. To avoid any carry-over or habituation effects, only one dummy per day was presented in the respective territory. As the order of presentations might also have an influence on the response, the order of dummy presentation was balanced between territories, i.e. every dummy type (species) was presented in two territories as first model, in two as second, in two as third and in two as fourth model. For each male territory owner, the following variables were collected: response latency, minimum distance to the dummy, number of flights over the dummy, time spent near dummy (<10 m).

Playback presentation

Playbacks were carried out in April 2008 and May 2009, using the conspecific song as well as heterospecific songs of *O. h. hispanica/melanoleuca*, *O. pleschanka*, and *O. finschii*. The latter served as some kind of control because the species is not closely related to the three other *Oenanthe* (Aliabadian et al. 2007), but a regular winter visitor to Cyprus. All stimuli were standardised on 30 s in 2008 and to 60 s in 2009. The calls for 2008 were obtained from different commercial sound recordings (Roche 1995; Kosmos-Verlag 2002; Schulze 2003), and for 2009 created from own recordings of *O. cypriaca* from 2008 (by C.R.),

Table 1 Taxa sequenced in this study

Taxon	Collection code[b]	GenBank accession number	Origin
Oenanthe cypriaca[a]	51654	HM126495	Cyprus
Oenanthe cypriaca[a]	51655	HM126496	Cyprus
Oenanthe cypriaca[a]	51656	HM126497	Cyprus
Oenanthe cypriaca[a]	51658	HM126498	Cyprus
Oenanthe cypriaca[a]	51660	HM126499	Cyprus
Oenanthe cypriaca[a]	51662	HM126500	Cyprus
Oenanthe cypriaca[a]	51664	HM126501	Cyprus
Oenanthe cypriaca[a]	51666	HM126502	Cyprus
Oenanthe cypriaca[a]	51670	HM126503	Cyprus
Oenanthe hispanica	53444	HM126504	Niger
Oenanthe hispanica	53445	HM126505	Mali
Oenanthe hispanica	53446	HM126506	Mali
Oenanthe hispanica	53448	HM126507	Mali
Oenanthe hispanica	53449	HM126508	Mauritania
Oenanthe hispanica	53451	HM126509	Mauritania
Oenanthe hispanica	53452	HM126510	Mauritania
Oenanthe hispanica	53453	HM126511	Mauritania
Oenanthe hispanica	53454	HM126512	Mauritania
Oenanthe hispanica	53455	HM126513	Mauritania
Oenanthe hispanica	53456	HM126514	Mauritania
Oenanthe hispanica	53457	HM126515	Mauritania
Oenanthe hispanica	53467	HM126516	Morocco
O. hispanica melanoleuca	53468	HM126517	Israel
O. hispanica melanoleuca	53469	HM126518	Israel
Oenanthe pleschanka	53458	HM126519	Kenya
Oenanthe pleschanka	53459	HM126520	Kenya
Oenanthe pleschanka	53460	HM126521	Kenya
Oenanthe pleschanka	53461	HM126522	Kenya
Oenanthe pleschanka	53462	HM126523	Kenya
Oenanthe pleschanka	53463	HM126524	Kenya
Oenanthe pleschanka	53464	HM126525	Kenya
Oenanthe pleschanka	53465	HM126526	Kenya
Oenanthe pleschanka	53466	HM126527	Kenya
Oenanthe deserti		GQ482258	Mugur Aksy, Russia
Oenanthe pleschanka		GQ482271	Kazakhstan
Oenanthe pleschanka		GQ482272	Mongolia
Oenanthe pleschanka		GQ482273	Mongolia
Oenanthe pleschanka		GQ482274	Novorossiysk, Russia
Oenanthe pleschanka		GQ482275	Mugur Aksy, Russia
Oenanthe oenanthe		AY666389	Canada
Oenanthe oenanthe		DQ433051	Iceland
O. picata picata		DQ683509	Iran

Table 1 continued

Taxon	Collection code[b]	GenBank accession number	Origin
Oenanthe leucopyga		DQ683508	Morocco
Oenanthe pleschanka		DQ683507	Iran
Oenanthe pleschanka		DQ683506	Iran
O. oenanthe seebohm		DQ683505	Morocco
O. oenanthe libanotica		DQ683504	Iran
O. oenanthe libanotica		DQ683501	Iran
O. moesta moesta		DQ683500	Morocco
O. lugens persica		DQ683497	Iran
Oenanthe isabellina		DQ683495	Iran
O. hispanica melanoleuca		DQ683489	Iran
O. finschii barnesi		DQ683487	Iran
O. deserti homochroa		DQ683485	Morocco
O. deserti deserti		DQ683484	Iran
Oenanthe chrysopygia		DQ683481	Iran
Oenanthe alboniger		DQ683480	Iran
Saxicola insignis		GQ482619	Mongolia
Saxicola maura		GQ482621	Zaliv, Russia
Saxicola rubetra		GQ482628	Russia
Saxicola rubicola		GQ482633	Akhmetovskaya, Russia
S. torquata axillaris		FJ657469	Kenya
Monticola gularis		GQ482168	Mongolia
Monticola saxatilis		GQ482172	Kazakhstan
Tarsiger cyanurus		GQ482758	Mongolia
Luscinia luscinia		DQ683476	Sweden
Luscinia megarhynchos		DQ683477	Iran
Phoenicurus erythronotus		GQ482382	Russia

[a] Sampled in breeding range. The O. h. hispanica and O. h. melanoleuca were sampled during migration and in their winter quarters

[b] The collection code corresponds to those of the Institute of Pharmacy and Molecular Biotechnology (IPMB), University of Heidelberg

and from recordings of O. h. melanoleuca from the British Sound Library. For every species, different stimulus tapes were created to avoid pseudo-replication (Kroodsma 1989). The calls were digitally edited to minimise disturbing noises and to erase songs or calls of other bird species using Avisoft SASLab Pro 4.3. Afterwards, the calls were copied to an analogous tape using a Grundig 437 CD player and AIWA CX-Z87M cassette recorder to produce the playback tapes. Calls were broadcast using a small portable Toshiba MCR 103 cassette recorder. The different playback tapes were standardised at ca. 65–75 dB measured at 1 m distance from the speaker using a digital sound-level meter with A-level weighting (PeakTech 5035). In 2008, there were $n = 8$ dif-

Fig. 1 Dummy models of the four types used for the experiments. From *left* to *right O. cypriaca*, *O. pleschanka*, *O. h. melanoleuca* (*dark*), and *O. h. melanoleuca* (*light*)

ferent playback stimuli available for *O. finschii*, $n = 9$ for *O. pleschanka*, $n = 17$ for *O. h. hispanica/melanoleuca*, and $n = 8$ for *O. cypriaca*. The *O. hispanica* playbacks were from both subspecies, *O. h. hispanica* and *O. h. melanoleuca*, and from populations that had not been assigned subspecific status. In 2009, there were $n = 15$ playback tapes for *O. cypriaca*, and $n = 13$ for the eastern subspecies *O. h. melanoleuca*. During every trial, the focal individual received two playbacks—one conspecific and one heterospecific (matched pair samples). However, to avoid carry-over effects in playback designs (den Hartog et al. 2007), in half the trials the conspecific stimulus was played first followed by the heterospecific song, and in the other half it was vice versa. Responses to the playback tapes were coded in the following manner: 0 = no visible or acoustic response, 1 = weak response (changing/altering of song, calling, or dipping, but no approach), 2 = medium response (approaching the speaker but less than 10 m, or 1–2 flights in the direction of the speaker), 3 = strong response approaching the speaker up to 10 m at least once), 4 = very strong response (two approaches nearer than 10 m or minimum distance below 5 m).

Statistical analysis

For the playback and dummy presentation, non-parametric tests were used: Wilcoxon-test for the comparison of two dependent observations, and Mann–Whitney U test for two independent observations. To compare more than two different treatments, Friedman test for dependent and Kruskal–Wallis for independent observations were used.

Further, the responses to the dummy presentation were subjected to a factor analysis to create a single response variable. Three variables (minimum distance, overflights, time spent less than 10 m near the dummy) were $\log_{10}$-transformed after the addition of one. Afterwards, these variables and response latency were z-transformed and subjected to a principal component analysis with varimax rotation.
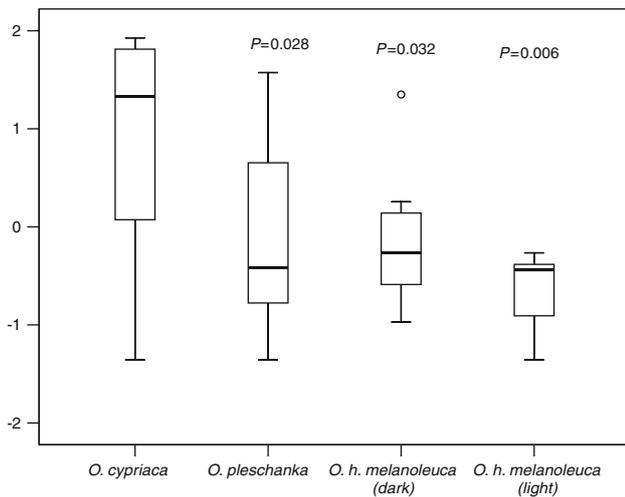
Results

Phylogenetic analysis

We obtained 679 base pairs (bp) of the mitochondrial cytochrome c oxidase subunit 1 (*CO1*) gene. ML and BI analyses support the close relationship between *O. cypriaca*, *O. pleschanka* and *O. h. melanoleuca* (Fig. 2). With a relative high posterior probability value (0.98), *O. cypriaca* clusters closer to migrating *O. h. melanoleuca* from Iran and Israel and *O. pleschanka* from Iran than to *O. pleschanka* obtained from Kazakhstan, Russia and Mongolia and wintering areas in East Africa (Kenya). Uncorrected p distance values between *O. cypriaca* and *O. pleschanka* from Kenya consist of 0.5%. *O. hispanica* samples from different West African wintering localities such as Mali, Morocco, Niger and Mauritania cluster separately in different branches of the tree (Fig. 2). The birds, which winter in these areas and belong phenotypically to the sub-species *O. h. hispanica*, are probably of Central and Western Mediterranean origin (e.g. Italy, France and Spain). The phylogenetic relationships among other *Oenanthe* species at the base of the tree have been previously recovered and discussed in Aliabadian et al. (2007) and Outlaw et al. (2010).

Dummy presentation

Friedman tests indicated significant differences in the response of *O. cypriaca* males towards the four different

Fig. 2 Bayesian tree based on
679 bp of the *co1* gene.
Posterior probability values
(>0.90) are indicated for each
node. The origin of samples is
indicated in Table 1

models in minimum distance to the dummy ($\chi^2 = 8.52$, $df = 3$, $P = 0.036$), time spent < 10 m from the dummy ($\chi^2 = 12.67$, $df = 3$, $P = 0.005$), and number of overflights ($\chi^2 = 16.63$, $df = 3$, $P = 0.001$). Response latency did not differ significantly ($\chi^2 = 1.75$, $df = 3$, $P = 0.625$). The PCA produced one factor with an Eigen-value greater than one (Eigen-value: 2.93; 73.3% variance explained). Factor loadings were −0.96 concerning minimum distance, 0.88 time spent near the dummy, 0.88 (overflights), and −0.67 concerning response latency. *O. cypriaca* responded stronger towards the conspecific model (Fig. 3) than to the *O. pleschanka* model ($T = 2.77$, $df = 7$, $P = 0.028$), the

Fig. 3 Responses of *O. cypriaca* towards four dummy presentations (stimuli; based on a PCA, see "Methods"). Significance is indicated between the responses towards the conspecific and each of the three heterospecific dummies

dark-throated *O. h. melanoleuca* model ($T = 2.67$, $df = 7$, $P = 0.032$) and the light-throated *O. h. melanoleuca* model ($T = 3.92$, $df = 7$, $P = 0.006$). However, responses did not differ significantly between the three heterospecific models ($P > 0.12$; Fig. 3).

Playbacks

In the matched pair comparisons, *O. cypriaca* responded stronger to conspecific playbacks (Table 2) than to heterospecific playbacks of *O. h. hispanica/melanoleuca*, *O. pleschanka* and *O. finschii*. Responses to playbacks were significantly different between the four stimuli (Kruskal–Wallis-Test: $\chi^2 = 20.40$, $df = 3$, $P < 0.001$; based on the first playback for every individual/trial; Fig. 4). In May 2009, for the heterospecific playbacks, only songs of the *O. h. melanoleuca* were used because the response in 2008 was strongest towards *O. h. hispanica/melanoleuca*. Similarly to 2008, *O. cypriaca* reacted most strongly towards conspecific playbacks (Table 2), and in only 2 out of 15 cases did a response towards *O. h. melanoleuca* playback occur. The response towards playbacks was weaker in May 2009 than in April 2008.

Discussion

The results of the behavioural experiments support the pre-zygotic isolation of *O. cypriaca* towards *O. pleschanka* and *O. h. melanoleuca* because *O. cypriaca* responded significantly more strongly towards conspecific playback and dummy presentations than to heterospecific stimuli.

Table 2 Responses of *O. cypriaca* males towards playback presentations

	n	*Z*	*P*
2008			
Stimulus			
O. pleschanka			
Negative ranks	12	−2.591	0.010
Positive ranks	2		
Ties	3		
Total	17		
O. hispanica (both subspecies and undetermined populations)			
Negative ranks	11	−2.249	0.025
Positive ranks	3		
Ties	1		
Total	15		
O. finschii			
Negative ranks	3	−1.633	0.102
Positive ranks	0		
Ties	0		
Total	3		
2009			
O. h. melanoleuca			
Negative ranks	6	−2.050	0.040
Positive ranks	1		
Ties	8		
Total	15		

Statistics based on Wilcoxon test for matched pairs (details see "Methods")
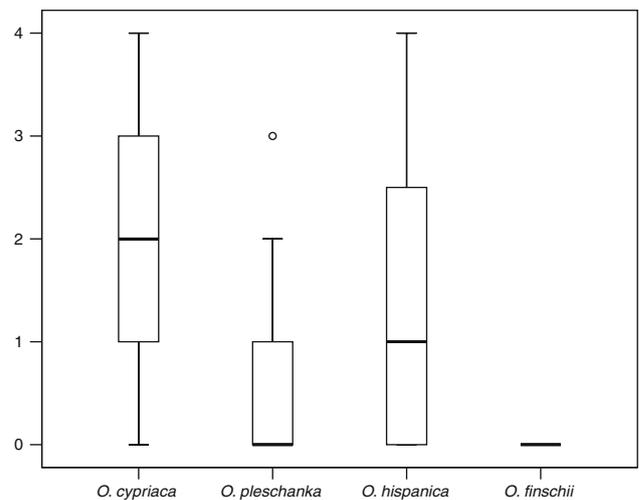
Fig. 4 Responses of *O. cypriaca* towards conspecific and different heterospecific playbacks. *0* no response, *1* weak response, *2* medium response, *3* strong response, *4* very strong response. Data from 2008, *O. hispanica* playbacks from different and unknown subspecies (see "Methods")

Responses to playbacks are generally seen as a useful tool in avian systematics but must be treated with some caution when considering allopatric taxa. It is to be expected that a male reacts more strongly to a visual or vocal stimulus that it has been imprinted on than to an alien stimulus (e.g. an alloptric taxon). However, although the *Oenanthe* wheatears are allopatric with regard to their breeding area, *O. h. melanoleuca* is a regular spring migrant on Cyprus (Randler and Crabtree 2010). Therefore, the species are familiar with each other and cannot be considered as clearly allopatric. Further, as *O. cypriaca* is migratory there might be some overlapping in migratory (Israel) and wintering areas (Kenya), thus the heterospecific recognition should be at least partially developed. This makes the situation of *O. cypriaca* different from many other island endemics, especially from tropical archipelagos and from Macaronesia because those are sedentary species. *O. cypriaca* might be allopatric from the breeding range but is sympatric with related taxa considering winter quarters. However, detailed studies of the coexistence of the *Oenanthe* species on migration and in the winter quarters are scarce (Leisler et al. 1983; Randler and Crabtree 2010). Nevertheless, it is remarkable that this island population seems to maintain its integrity despite the opportunity to mix with mainland populations during their life cycle which strongly supports a pre-zygotic barrier.

From the molecular viewpoint, we are unable to clearly separate *O. cypriaca* from *O. pleschanka* and *O. h. melanoleuca*. The small genetic distance is in agreement with intra-population divergence in many other passerines (Alström et al. 2007, 2008), and this contradicts rather than supports the species status of *O. cypriaca*. Another interesting aspect is the non-monophyly which is strongly supported by mitochondrial data (Olsson et al. 2005). The molecular tree surprisingly indicates that migrating *O. h. melanoleuca* from Israel and *O. pleschanka* from Iran clustered together with *O. cypriaca*, which might be an indicator for the retention of ancestral polymorphism or introgression. There seems to be gene flow between *O. cypriaca* and *O. h. melanoleuca*, and between *O. h. melanoleuca* and *O. pleschanka* (Aliabadian et al. 2007). The latter, however, is well-known (Panov 2005). Gene flow between *O. cypriaca* and *O. h. melanoleuca* might be a result of introgression, although there is no firm evidence of this. Introgression may have occurred at different temporal stages, and it could be either recent or have taken place a long time ago (see below). An alternative hypothesis for the pattern might therefore be retention of ancestral polymorphisms.

Hybridisation is likely among *Oenanthe* species, especially between *O. pleschanka* and *O. h. melanoleuca* (Panov 2005; Aliabadian et al. 2007; Randler 2008), which show a wide hybrid zone in Iran. Although there are no breeding records from *O. cypriaca* outside Cyprus, it may

be possible that the species also breeds on the southern coast of Turkey, because the species apparently has some vagrancy potential (Förschler et al. 2010) and, recently, we have suggested probable breeding of *O. h. melanoleuca* on Cyprus (Randler and Crabtree 2010), which may also facilitate hybridisation between the taxa.

We used different measurements to assess the taxonomic status of *O. cypriaca* (see Alström et al. 2007, 2008), and we have to conclude that the mitochondrial data do not support species status of *O. cypriaca*, and even not of *O. h. melanoleuca* and *O. pleschanka*, while, surprisingly, the data support different forms within the western *O. h. hispanica* clade. Alström et al. (2008) discuss the limits of assigning species rank in allopatric taxa. Under a 'morphological' species definition, *O. cypriaca* might be a distinct species (Christensen 1974; Aliabadian et al. 2007; Förschler et al 2010), while under a 'phylogenetic' species definition it might not. Under a 'biological' species definition, the taxonomic status of *O. cypriaca* seems clearer because the song of *O. cypriaca* is different compared to the other three forms (Sluys and van den Berg 1982; Bergmann 1983). Despite the mitochondrial evidence against species status, we propose species status for *O. cypriaca* because of the behaviour towards dummies and playbacks, the strikingly different song (Bergmann 1983), and different morphometrics (Kaboli et al. 2006). *O. cypriaca* differs from *O. pleschanka* in 14 characters of external morphology (Förschler et al. 2010). Given this evidence, we suppose that *O. cypriaca* will maintain its genetic and phenotypic integrity in the future, which should be a significant criterion for assigning species rank (Helbig et al. 2002). An alternative would be to treat *O. pleschanka*, *O. h. melanoleuca* and *O. cypriaca* as populations of one single species. The results emphasise the importance of dense taxon sampling in intrageneric phylogenetic studies as requested by Olsson et al. (2005), and, we therefore suggest further sampling of the *hispanica–melanouleuca–cypriaca–pleschanka* complex in all breeding areas.

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