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Effects of Post-Glacial Range Expansions and Population Bottlenecks on Species Richness

BY

ANDERS ÖDEEN



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Abstract

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This thesis relates modern speciation theory to the effects of sudden changes in the range and size of populations. Special reference is made to the climatic oscillations during the Quaternary ice ages. A meta-analysis of laboratory experiments showed that support for allopatric speciation is weak, especially for the peripatric type of allopatric speciation. Furthermore, factors traditionally believed to increase the likelihood of speciation have had little effect on the generation of reproductive isolation in speciation experiments. However, the method of testing reproductive isolation appeared important, in the sense that experimentally derived sister populations were likely to demonstrate reproductive isolation from each other but not from the unaffected mother population. Raw data from mating tests showed that the poor isolation between mother and daughter populations was an effect of asymmetric mate preferences towards males from the mother population. This suggests that peripatric speciation can be effective in generating reproductive isolation between sister populations. The proposed mechanism is that males become less attractive to females by losing certain secondary sexual traits during population bottlenecks, and that females shift their preferences towards other male traits. Support for this mode of speciation is found in the widespread bird genus *Motacilla* (wagtails). This genus is characterised by extensive plumage variation and contains a large number of widely distributed taxa in the northern parts of its distribution. This thesis shows that taxonomic diversity of wagtails is inversely related to complexity in song and to diversity in molecular and mitochondrial markers. The northern taxa seem to be descendants of southern populations, which were subjected to bottlenecks during expansions into re-opened habitats after the last ice age. The bottlenecks would not only reduce genetic diversity but also inhibit cultural transmission of song to the leading edge of colonisers, allowing sexual selection on other traits, such as plumage. Rapid plumage differentiation among wagtail taxa appears to be a recurrent process and has led to convergent evolution, making the currently recognised species *Motacilla flava* (Yellow Wagtail) polyphyletic.

Key words: Peripatric speciation, bottlenecks, mating asymmetries, Kaneshiro model, Quaternary ice-ages, phylogeny, *Motacilla*, *Motacilla flava*.

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- I** Florin, A.-B. & Ödeen, A. Laboratory environments are not conducive for allopatric speciation. *Journal of Evolutionary Biology*. In press.
- II** Ödeen, A. & Florin, A.-B. The Kaneshiro model revisited. Submitted.
- III** Florin, A.-B. & Ödeen, A. A new role for sexual selection in speciation. Submitted.
- IV** Ödeen, A. Radiation, loss of sexual traits and convergent evolution in yellow wagtails (*Motacilla flava* L). Manuscript.
- V** Ödeen, A. & Alström, P. Evolution of secondary sexual traits in wagtails (genus *Motacilla*). Manuscript.

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“Time flies like an arrow

Fruit flies like a banana”

Ulf Henriksson

INTRODUCTION

Speciation as a discipline has a special place in modern biology as it links so many topics in ecology, systematics and genetics. Speciation theory, as most issues in evolutionary biology and ecology, began with Darwin (1859). By formulating a plausible theory on how species change he paved the way for the scientific study of speciation. The next big step was taken with the “Modern Synthesis” (e.g. Dobzansky, 1937) when Darwin’s evolutionary theory was fitted into mendelian genetics. Up until modern times the thinking of the Modern Synthesists have governed most research on speciation, much because they formulated the still most widely supported definition of what species is, i.e., the Biological Species Concept or “BSC” (Mayr, 1942; Dobzhansky, 1937). The BSC became possible to formulate when the early modern syntesists demonstrated how genetically close populations can accumulate so called ”complementary genes” that have no deleterious effects within the population, but cause hybrid sterility or inviability when combined with different extrapopulation complementary genes (Orr, 1995). According to the BSC, the taxonomic rank of natural groups is judged on basis of their degree of integration (in sympatry or parapatry) and, in cases of geographical isolation (allopatry), the degree of differentiation that is assumed to serve as barriers to reproduction in zones of contact. Unfortunately, for allopatric populations, this will be a judgement based on circumstantial evidence. One has to assume that reproductive isolation and morphological differentiation always show correlated variation and evolve at an equal rate in contact zones and in allopatry.

Models of speciation

The definition of species as reproductively isolated groups of organisms was paralleled by speciation theories where isolation was believed to be the causal agent. Allopatric speciation, where new species arise from geographically isolated populations of the same ancestral species, is the most widely accepted of all current speciation models (Mayr, 1942, 1963; Lynch, 1989; Coyne, 1992; Rice & Hostert, 1993). It can be divided into vicariance and peripatric (or “peripheral isolates”) speciation (Lynch, 1989) depending on the location of the geographical split and the size of the sub-populations. In vicariance speciation (“the dumbbell model” in Mayr, 1982), a continuous population is split in the centre of its distribution, giving rise to two or more large, isolated sub-populations. With time the

sub-populations are thought to evolve reproductive isolation as a by-product of genetic drift and /or divergent selection pressures.

An often-discussed phenomenon in speciation theory is the occurrence of many unique species on islands. This has led several authors to theorise about the possibility that small peripheral populations, like those on islands, evolve faster than a large population. Since vicariance speciation requires a large amount of genetic differentiation to lead to reproductive isolation in secondary contact, the process is generally believed to be rather slow. Reproductive isolation could evolve somewhat faster (involve fewer genetic changes) in peripatric speciation (Mayr, 1954). Here a small peripheral portion of the population becomes isolated and may undergo one or several (Carson, 1975) bottlenecks. Genetic drift caused by low population size during the bottlenecks together with relaxed selection pressure under the following flush phase, when the population rapidly increases in size, allows the formation of new gene combinations that would not have survived in the original population. Reproductive isolation is then believed to evolve either as a by-product of the genetic changes (Mayr, 1954; Carson, 1975; Templeton, 1980) or as a consequence of relaxation of mating preferences in bottlenecks (Kaneshiro, 1989). According to another development of the allopatric speciation theory, the reinforcement theory (Dobzhansky, 1937), even small genetic differences acquired in isolation might lead to reproductive isolation in secondary contact if the production of hybrids with low fitness selects for prezygotic isolation.

The last half century of speciation research has followed the lead of the modern synthesists and concentrated theory and empirical investigation on the role of isolation in limiting gene flow and promoting genetic drift. In recent years, however, cases have been found of extremely rapid divergence with very little genetic differentiation, much less than what is needed for allopatric speciation (eg. East African cichlids; Meyer, 1993; Seehausen & van Alphen, 1998, and Bowerbirds; Uy & Borgia, 2000). Furthermore, new theoretical and empirical work has increased the plausibility of speciation without geographical isolation (sympatric speciation). This has motivated a shift of focus away from isolation and towards the very mechanisms that initiate divergence. The old geographical classification of speciation modes is no longer taken for granted (Schluter 2001; Via 2001). The concept of ecological speciation, where isolation is an effect of adaptation to divergent selective regimes, has gained consistent support from empirical evidence (Schluter, 2001). Without divergent selection, even weak natural selection on female preference may hinder

developing disparate mating traits and reproductive isolation from drift (Turelli *et al.*, 2001).

Sexual selection can lead to rapid divergence between populations since it directly affects the traits involved in reproductive isolation and increases the overall rate of change (Panhuis *et al.*, 2001). This has spurred an interest among biologists to examine the possible role of sexual selection in speciation. Sexual dichromatism and plumage ornamentation in birds (Barraclough *et al.*, 1995; Møller & Cuervo, 1998) and polyandry (increased potential for sexual selection) in insects (Arnqvist *et al.*, 2000) have been shown to correlate with species richness. Furthermore, prezygotic (sexual) isolation seems to evolve faster than postzygotic isolation (Coyne & Orr, 1997).

Nevertheless, in the phylogenies of many animal groups, the traits presumably maintained by sexual selection are more often lost than gained (Wiens, 2001). This paradoxical fact puts a spoke in the wheel of most current models of speciation by sexual selection. The Kaneshiro model (Kaneshiro, 1976, 1980), however, directly relates to loss of sexually selected traits. This model is a development of peripatric speciation with a special focus on sexual selection. It proposes that sexual selection might act as a stabilising agent on species-specific mating under normal circumstances and that speciation would appear as a direct consequence of relaxed sexual selection pressures following bottlenecks. Opportunities for the evolution of new mating traits come during population bottlenecks when parts of ancestral male secondary sexual traits (e.g. courtship behaviour) are thought to be lost. As a consequence, females will be selected to accept less extravagant courtship display. The result will be that females of ancestral populations discriminate against males from derived populations while females from derived populations accept ancestral males. Such patterns of asymmetric pre-mating isolation have been observed many times, mostly in insects (Giddings & Templeton, 1983; Kaneshiro, 1983, 1989; Shaw & Lugo, 2001), but also in birds. In a study of house finches (*Carpodacus mexicanus*) females of all populations were shown to prefer the ancestral male type, which is the type with the largest and most brightly coloured ventral patch (Hill, 1994).

Support for speciation theories

Although complete reproductive isolation is a prerequisite for speciation under the BSC (Mayr, 1942; Dobzhansky, 1937), it has only been reported from one laboratory experiment (Thoday & Gibson, 1962). In general, the

published records on laboratory speciation experiments have yielded equivocal results (Rice & Hostert, 1993; Ödeen & Florin, 2000), implying that there are certain conditions required for speciation that have not been fulfilled in these studies. When trying to evaluate the empirical evidence for different modes of speciation most authors have performed narrative studies. However, we need not only count the number of studies that statistically deviate from random mating but quantify the actual degree of assortative mating achieved. This is important, since all studies are not equally reliable due to differences in sample size. Meta-analysis procedures take sample size into account by quantitative weighting (Arnqvist & Wooster, 1995). Meta-analysis is hence a powerful tool to determine the true, underlying, size of the effect speciation experiments have had on the evolution of assortative mating. Another prerequisite for speciation according to the BSC is that once reproductive isolation has been achieved it must be permanent (Moya *et al.*, 1995). Therefore, to separate speciation from random fluctuations in mating behaviour it is necessary to demonstrate consistency in reproductive isolation over generations.

The patterns of asymmetric pre-mating isolation thought to result from Kaneshiro's (1976, 1980) sexual model have been observed many times, mostly in insects (Giddings & Templeton, 1983; Kaneshiro, 1983, 1989; Shaw & Lugo, 2001), but also in birds. In a study of house finches (*Carpodacus mexicanus*) females of all populations were shown to prefer the ancestral male type, which is the type with the largest and most brightly coloured ventral patch (Hill, 1994). Kaneshiro's speciation model, however, has also been met with criticism. Meffert & Bryant (1991) found that female discrimination in the housefly *Musca domestica* was not related to reduction in male courtship complexity and questioned the power of Kaneshiro's model to explain the commonly observed mating asymmetries in nature and laboratories. Alternative models that do not presuppose simplified courtship behaviour have challenged Kaneshiro's theory. Wasserman & Koepfer (1977) argued that reinforcement might explain mating asymmetries observed when mainland and island populations meet. Because mainland females are more likely than island females to be sympatric to males of other species, they are also more likely to produce maladaptive hybrids and hence are selected to be more discriminating. Other explanations for mating asymmetries include inbreeding depression in the derived population (see Mooers *et al.*, 1999), or scarcity of suitable mates (Wirtz, 1999).

There are also peripatry studies where mating asymmetries have been found to be altogether absent (Markow, 1981; Galiana *et al.*, 1993) or even going

in a direction opposite (Watanabe & Kawanishi, 1979) to that predicted by Kaneshiro (1976, 1980). In defence of the Kaneshiro model, Giddings & Templeton (1983) and Kaneshiro (1983) have pointed out that the theory applies only to allopatric populations that have been bottlenecked and not selected, and that the examples where the predictions have not been supported do not fulfil these requirements.

Although there is ample evidence corroborating Kaneshiro's hypothesis, this is mostly based on "male-choice" experiments. In these, a male is given the choice between a female of his own population and a female of another. This means that the females have not been allowed to choose at all, and hence it is impossible to distinguish whether females of the derived population are mating randomly or if they actually prefer males from the ancestral population. It is of fundamental importance to distinguish between these different female behaviours; lowering the threshold for female acceptance, but still preserving a preference for the ancestral trait, will merely limit the intensity of stabilising sexual selection on the pre-existing male secondary sexual characters and not set the stage for speciation between a bottlenecked population and its ancestor. Furthermore, in many studies of mating asymmetries in wild populations the designations of derived and ancestral populations are questionable. Laboratory populations, where the ancestral relationships are known with certainty, are more reliable tests of the Kaneshiro model.

Quaternary ice ages

Many Palearctic and Nearctic species form patchworks of distinct geographic variants. In recent years, several attempts have been made to explain their origin in the light of the major climatic changes caused by the cyclic glaciations of the Pleistocene epoch (Hewitt, 2000). During the peak of the last ice age steppe and tundra covered most of central Europe, Asia and North America, while the northern parts and the major mountain chains were covered in ice. Hence, as most of the Northern Hemisphere would have been inhabitable, the ancestors of modern populations must have taken refuge in the south. Populations in separate regions would have evolved in isolation for many tens of thousands of years, coming into contact only during range expansions in the relatively brief, warmer, interglacial periods. These cycles of long isolation followed by secondary contact fit well into plausible scenarios of allopatric divergence and speciation.

Although genetic differences seem to have accumulated primarily during isolation in glacial refugia, recent evidence suggests that very rapid phenotypic changes forming new recognisable taxa could have occurred when the ice retreated (Hewitt, 2000). Since there must have been very little time for genetic divergence, a shift of focus from the traditional issues of reproductive isolation to the mechanisms that initiate divergence (Via, 2001) might be productive when investigating such radiations. Hence, there is a role in post-glacial re-colonisation theory for selection speciation, including the Kaneshiro model. Following the relatively rapid warming at the end of the Pleistocene, leading edge expansion – a chain of colonisations by a small part of the population – probably subjected the northerly immigrants to severe bottlenecks followed by enormous flushes (Merilä *et al.*, 1997; Kvist *et al.*, 1999). In this process males may have lost attractive ancestral traits and females would then have been selected to lower their acceptance threshold and mate with less attractive males. The flush would hence act as a respite from stabilising selection on ancestral male phenotypes, allowing the development of new male traits, which could be subjected to sexual selection and become fixed (Kaneshiro, 1989).

METHODS AND MATERIAL

Quantitative review and meta-analysis

The basic design in allopatric speciation experiments consists of spatially isolating populations of *Drosophila* and *Musca* flies, taken from the same, often outbred, stock. After a number of generations in isolation, mating tests are performed to discern the amount of reproductive isolation achieved between experimental populations or to the original stock. Published data from surveys of the literature since the 1950's and raw data provided by the authors were analysed. Raw data was also provided from one unpublished study (Jones, Widemo & Arrendal in prep.). Studies were used only if populations had been kept apart uninterruptedly and if the number of matings in different choice situations had quantified prezygotic isolation.

The experiments were first separated according to the method by which isolation tests were made: *Derived* – two daughter (derived) populations tested against each other, *Origin* – a derived population tested against the ancestral, unselected, unbottlenecked stock. This was done to avoid pseudo-replication, as some populations occurred in both groups. Some derived populations had been tested against several others. Again, to avoid pseudo-replication, independent combinations were chosen at random. Then the

experiments were divided according to the allopatric speciation model tested: *Vicariance* or *Peripatric* speciation. Third, these groups were divided with respect to selection regime. Experimental populations where no selection, or parallel selection, was applied were classified as *Drift*, and experiments with divergent selection, for example for high and low bristle number, were classified as *Selected*. This subdivision resulted in four speciation model categories: *Vicariance–Drift*, *Vicariance–Selected*, and *Peripatric–Drift*. The fourth combination, *Peripatric–Selected*,

For each experiment we noted or calculated the following variables: reproductive isolation at the end of the experiment (Y) (Bishop *et al.*, 1975), the total number of matings in reproductive isolation tests (sample size), effective population size (N_e), the number and size of bottlenecks, and the duration of the experiment in number of generations. To separate chance fluctuations in Y from true reproductive isolation, we also noted the value of Y from the penultimate mating test (Y_p), which was usually performed around the middle of the ongoing experiment.

The relationship between Y (the effect size) and the observed number of matings (sample size) was examined. A “funnel graph”, i.e. a plot of sample size against the effect size, can reveal the true effect of the tested variable (Palmer, 2000). It can also reveal the existence of publication bias, in which case the effect size becomes correlated to the sample size. The effect size is in meta-analyses normally weighted by the inverse of its variance (Osenberg & StMary, 1998) but the variance could not be calculated. Therefore, sample size was used as the most appropriate weighing variable available.

For tests of mating asymmetry, three different types of mate choice were acknowledged: *Female choice*, where one female was given the choice between a male from a different population and a male from her own, *Male choice*, where one male was given a similar choice between two females, and *Multiple choice*, where several females and males from two populations were allowed to choose simultaneously. The types of mating were quantified and denoted by the following variables: XX , XY , YY , and YX . X represents the derived population and Y the ancestral population, with females given first (e.g. XY represents derived females mating with males from the ancestral population).

Study species

Apart from reviews of experiments using *Drosophila* and *Musca* flies, the study organisms of choice for this thesis belong to species and subspecies of wagtails (Aves: Passeriformes: Motacillidae: *Motacilla* L). Wagtails (*Motacilla*) are a passeriform genus, placed together with the genera *Anthus*, *Macronyx* and *Hemimacronyx* and the monotypic *Tmetothylacus* and *Dendronanthus* in the family Motacillidae (Mayr & Greenway, 1960; Voelker & Edwards, 1998). Wagtails are small insectivorous birds, widely distributed across Eurasia and Africa. Some of the species are very well known and popular among the general public owing to their conspicuous plumages and commonness near human habitations. The taxonomy is highly debated due to regular interbreeding between several taxa (review in Alström *et al.*, in press). All wagtails are fairly similar in size and structure, while their plumages are highly divergent, especially in temperate regions. In the northerly distributed White wagtail (*M. alba*) and Yellow wagtail (*M. flava*) species complexes the plumage differentiation between subspecies is exceptionally high – comparable to that between wagtail taxa considered to be separate species. Contrary to plumage, songs are most diverse and complex in southerly-distributed taxa. All species occur in open habitats, and some are mainly or exclusively found close to water. Most wagtails breed in the plains, but some taxa are also found at high altitude. Distributions are often wide but generally non-overlapping. Despite the large number of currently recognised taxa (39), no local area holds more than four during the breeding season.

The Yellow wagtail (*Motacilla flava* L) complex consists of a large number of subspecies (thirteen in the latest taxonomy: Alström *et al.*, in press), all with a parapatric distribution (only limited overlap) of breeding ranges mostly within the glacially disturbed regions of the Northern Hemisphere (Fig 1). Despite being the focus of many decades of intensive interest and research, the Yellow wagtail provides today one of the best-known examples of complex and unresolved intraspecies relationships. The species is perhaps best known for its extreme diversity in male plumage coloration between subspecies. In all subspecies, the summer adult male is olive-green on the back and bright yellow on the underside. The head coloration, however, varies distinctly between grey in different shades, black, white, and yellow to green. Above the eye, many subspecies have a broad or narrow supercilium in yellow or white. In a few subspecies, the chin is white instead of yellow. Apart from some north to south variation in location call types (Czikeli, 1985), male head plumage is virtually the only trait distinguishing the subspecies. The song in wagtails is generally

considered to be one of the simplest in the avian world (e.g. Mullarney *et al.*, 1999). It usually consists of one to three repeated syllables.



Figure 1 Breeding distributions of yellow wagtail subspecies. 1. *Motacilla flava thunbergi*, 2. *M. f. macronyx*, 3. *M. f. flava*, 4. *M. f. beema*, 5. *M. f. leucocephala*, 6. *M. f. tschuschensis*, 7. *M. f. iberiae*, 8. *M. f. cinereocapilla*, 9. *M. f. pygmaea*, 10. *M. f. feldegg*, 11. *M. f. flaviventris*, 12. *M. f. lutea*, 13. *M. f. taivana*. Drawings by Bill Zetterström. Reproduced from Alström *et al.*, in press, with permission from the publisher. Based on Alström, *et al.*, in press. However, the broad intergradation zone in Finland (1+3) reflects personal observations. Drawing by Kerstin Ödeen.

Specimens and genetic analysis

Samples were collected from live birds trapped with mist nets in breeding areas and migration locales in Europe, Asia and Africa. Blood (20 µl from the brachial vein in the wing) and/or a few breast or tail feathers were collected and the trapped bird was photographed together with the sample tube for later reference. The samples include almost the complete currently recognised taxonomy of wagtails (Alström *et al.*, in press). Two tissue samples were taken from dead birds and some samples were borrowed from museum collections. A Yellow-throated Longclaw (*Macronyx croceus*) was also sampled and one Meadow Pipit (*Anthus pratensis*), one *Passer montanus* and one *Prunella modularis* sample was borrowed from the Swedish Museum of Natural History.

Genomic DNA from feather bases, tissue and some blood samples was extracted using the DNAeasy Tissue Kit (Qiagen). Other blood-samples

were Chelex or Phenol-chloroform extracted using standard procedures. Three mitochondrial regions were analysed: (1) a segment running from the complete control region (CR) via tRNA-Phe into the 12S ribosomal RNA (12S) gene (45 bp), (2) the complete (1041 bp) NADH dehydrogenase subunit 2 (ND2), and (3) a 550 bp segment of mtDNA, including 128 bp of the 3'-end of ATPase 8 and 432 bp of the 5'-end of ATPase 6 (ATP8+6). Also analysed was a ~550 bp long intron in the chromo-helicase-DNA binding protein gene on the Z-chromosome (CHD-Z) (Fridolfsson and Ellegren 1999). Females (which have only one copy of the Z-chromosome) were primarily used in nuclear analyses. Twenty-one different primers were used, of which seven were designed for this study.

Fragments were amplified by Polymerase chain reaction (PCR) Of the PCR products 5 µl were run on agarose gels to check length and specificity of the amplifications and the remaining 20 µl were cycle sequenced in both directions with BigDye Terminator Ready Reaction kit (Applied Biosystems). The products were run on ABI-prism 310 and 377 automated sequencers. The resulting electropherograms were analysed with Sequencing Analysis 3.3 software (Applied Biosystems) and aligned and confirmed with AutoAssembler 2.1 software (Applied Biosystems). A 10 kbp PCR fragment spanning from tRNA-Glu to ATP6 was amplified with the Expand Long PCR Template System (Roche Diagnostics). The product was used as a template in a second PCR with primers flanking the control region to check for accidental amplification of nuclear copies of mitochondrial DNA.

Sequences were aligned by eye in Se-Al 1.0a1 (Andrew Rambaut; available from <http://evolve.zoo.ox.ac.uk/Se-Al/Se-Al.html>) or automatically using the program Clustal W version 1.7 (Thompson *et al.*, 1994) Phylogenies were estimated by maximum parsimony (MP) and maximum likelihood (ML) using PAUP* (Swofford, 2001) and by Bayesian inference using MrBayes (Huelsenbeck & Ronquist, 2001; Huelsenbeck & Ronquist, in press). The sequences were analysed separately and in different combinations. Yellow-throated longclaw *Macronyx croeus*, meadow pipit *Anthus pratensis*, tree pipit *A. trivialis*, dunnock *Prunella modularis* and house sparrow *Passer domesticus*, were used as outgroups.

Genetic structure was tested using AMOVA in Arlequin, v. 2.000 software (Excoffier *et al.*, 2000) in two ways. Statistical testing was done by randomisation in Arlequin. The pair-wise sequence comparison approach (mismatch distribution) by Rogers (1995) was used to test possible expansions as implemented in Arlequin (Excoffier *et al.*, 2000).

Vocal analysis

Tape recordings of song were taken in the field. To avoid bias from cultural transmission from males of other subspecies, only recordings from monotypic areas were used. The recordings were converted to sonagrams for visual comparison using Canary v. 1.2 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA).

RESULTS AND DISCUSSION

Empirical support for allopatric speciation ? (Paper I)

Allopatric speciation is the most widely accepted of all current speciation models (Mayr, 1942, 1963; Lynch, 1989; Coyne, 1992; Rice & Hostert, 1993) It can be divided into vicariance and peripatric (or “peripheral isolates”) speciation (Lynch, 1989) depending on the location of the geographical split and the size of the sub-populations. The vicariance model of allopatric speciation has been well proven empirically, while peripatric speciation has suffered severe criticism for being both implausible and empirically unsupported (Barton & Charlesworth, 1984; Barton, 1989, 1996; Coyne, 1992, 1994). The published records on laboratory experiments on speciation have yielded equivocal results (Rice & Hostert, 1993; Ödeen & Florin, 2000), implying that there are certain conditions required for speciation but not fulfilled in some of these studies.

A meta-analytic review of 25 publications of laboratory experiments on peripatric and vicariance allopatric speciation addressed the following three questions: 1) What was the true effect size of reproductive isolation? 2) Was the reproductive isolation persistent? 3) What influenced the development of isolation?

Contrary to popular belief, laboratory evidence for allopatric speciation is quite weak.

1) Laboratory allopatric speciation experiments have had little if any effect on reproductive isolation (Fig 2). The weighted mean reproductive isolation (Y) was not significantly different from the null hypothesis $Y = 0$, except in *Derived* tested *Vicariance* experiments ($t = 3.50$, $d.f. = 36$, $P = 0.001$)

2) Reproductive isolation against control populations was only intermittent. Y correlated with isolation in the penultimate mating test (Y_p) only in *Derived* types of tests (*Derived*: $r = 0.63$, $n = 18$, $P = 0.005$; *Origin*: $r =$

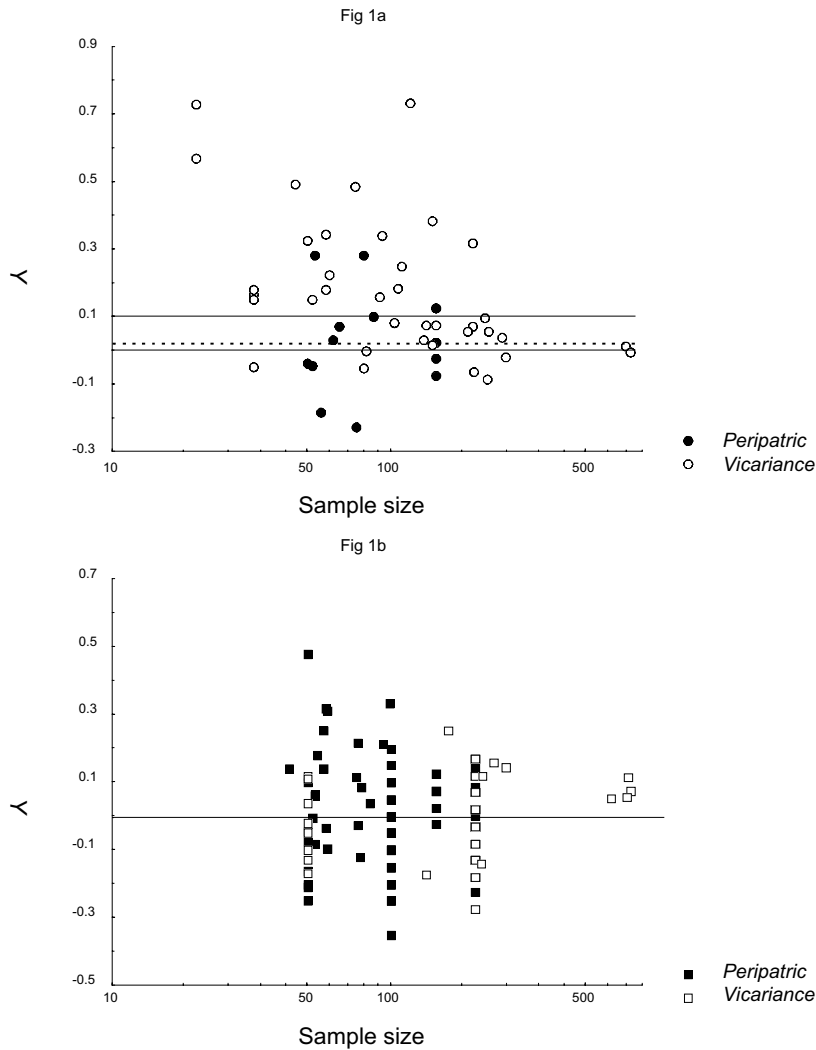


Figure 2. Reproductive isolation (Y) in published experiments with different number of matings observed (Sample size) to determine Y in mating tests between a) two derived populations (*Derived*) and b) one derived population and the original population (*Origin*). Peripatric speciation experiments (*Peripatric*) are shown with filled symbols and vicariance speciation experiments (*Vicariance*) with open symbols. The solid line denotes the null-hypothesis, namely random mating. The dashed line shows the weighted mean of Y for *Vicariance* and the dotted line for *Peripatric* experiments. In b), weighted mean Y for *Vicariance* and *Peripatric* experiments are too close to $Y = 0$ to be shown.

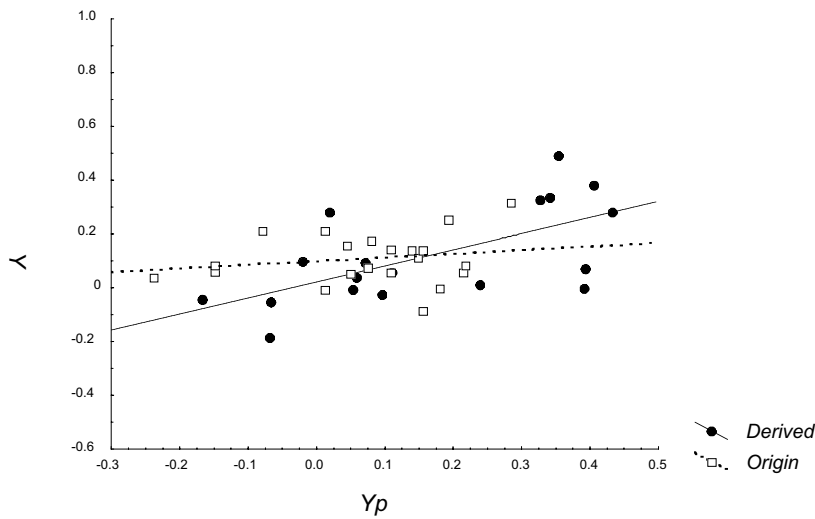


Figure 3. Reproductive isolation at the end of the experiment (Y) as a function of reproductive isolation in the penultimate test (Y_p) in mating tests between two derived populations (*Derived*, filled circles) and between one derived population and the original population (*Origin*, open squares). The solid line and the dashed lines show the weighted least square fits to *Derived* and *Origin* experiments, respectively.

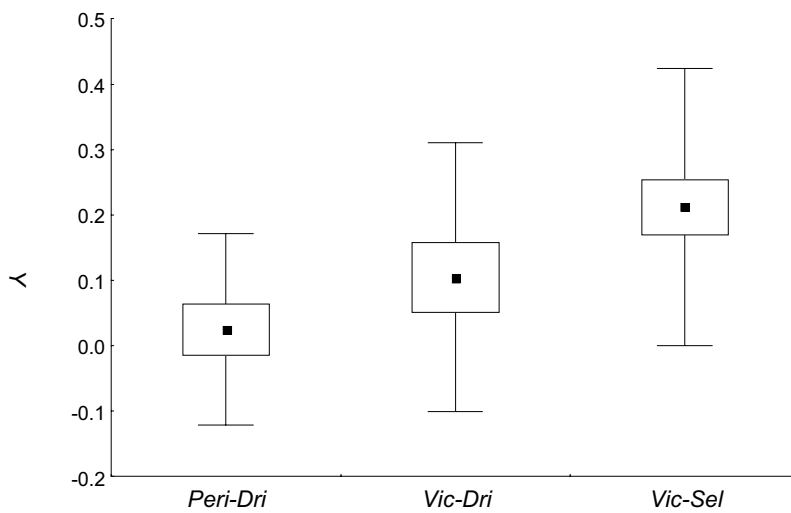


Figure 4. Reproductive isolation (Y) between derived populations in experiments testing different speciation models: vicariance speciation under divergent selection (*Vic-Sel*), peripatric speciation under drift (*Peri-Dri*), and vicariance speciation under drift (*Vic-Dri*). Means are shown together with standard errors (boxes) and standard deviation (whiskers).

0.20, $n = 21$, $P = 0.40$; Fig. 3). If experiments really promoted reproductive isolation, one would expect the slope of Y as a function of Y_p to be near or above one. The lack of correlation in *Origin* tested experiments suggests that all deviations from random mating in these experiments are due to chance fluctuations.

3) None of the investigated variables gave a fully satisfying explanation of the failure of laboratory experiment to demonstrate allopatric speciation. There was no effect of effective population size (N_e) on reproductive isolation (Y) in either *Derived* or *Origin* types of isolation tests (*Derived*: $r = -0.084$, $n = 43$, $P = 0.59$; *Origin*: $r = 0.12$, $n = 168$, $P = 0.13$). There was a correlation between Y and length of experiment but only among *Origin* tested *Vicariance* experiments (*Origin*: $r = 0.31$, $n = 52$, $P = 0.02$). This hints that speciation might have occurred, given enough time. Selection can also be important. It was found that divergently selected vicariance experiments (*Vicariance-Selected*) have been more successful than simple drift and peripatric experiments (Fig. 4; $F_{2, 48} = 3.74$, $P = 0.03$, $Y_{Vicariance-Selected} = 0.21 \pm 0.041$ SE, $Y_{Vicariance-Drift} = 0.10 \pm 0.053$ SE, $Y_{Peripatric-Drift} = 0.02 \pm 0.039$ SE).

This study gives some support to the vicariance model but no support to the peripatric model. However the results suggest that the way of testing isolation may influence reproductive isolation to a similar degree as the actual model of speciation tested (vicariance or peripatric) and much more than the selection regime. One reason could be that the total selection and/or drift that populations have been subjected to is twice as strong in *Derived* tests compared to *Origin* tests, where only the experimental population has been affected. A quite different possibility is that the evolution of reproductive isolation may be counteracted by adverse experimental conditions, which render individuals of the derived population less attractive with the result that both populations demonstrate mating preferences towards the unaffected control population (cf. Kaneshiro's mating asymmetry model (1976, 1980)).

Testing the Kaneshiro model (Paper II)

There is support for Kaneshiro's speciation model in the recurring phenomenon of asymmetric mating preferences between derived and ancestral species and populations, especially in the drosophilid fruitflies of the Hawaiian archipelago (see Giddings & Templeton, 1983 and Kaneshiro, 1983, 1989 for reviews). However, the evidence corroborating Kaneshiro's hypothesis is mostly based on "male-choice" experiments. In these, a male

is given the choice between a female of his own population and a female of another. Hence, females have not been allowed to choose at all, and hence it is impossible to distinguish whether females of the derived population are mating randomly or if they actually prefer males from the ancestral population. This distinction is important if the Kaneshiro model is believed to result in isolation between a founder population and its ancestor. If females of the bottlenecked population show a preference, however slight, for the ancestral male phenotype there should be stabilising selection on male mating characters rather than speciation through drastic alterations of the mating system. Therefore, to truly support Kaneshiro's model as a peripatric speciation model, females of a bottlenecked population should not show a preference for either male type. Another problem with many studies of mating asymmetries in wild populations is that the designations of derived and ancestral populations are questionable. Studies of laboratory populations, where the ancestral relationships are known with certainty, are more reliable tests of the Kaneshiro model.

A quantitative analysis of mating asymmetries based on raw data from published mate-choice experiments with laboratory populations was performed. The aim was 1) to see if discrimination of ancestral females against derived males is a general phenomenon, 2) to check whether derived female mating preferences are weaker than those of ancestral females as Kaneshiro's model predicts, 3) to see if such mating asymmetries are specific to peripatric situations, as has been claimed (Giddings & Templeton, 1983; Kaneshiro, 1983), and 4) to find out if there are other explanations for the observed mating asymmetries.

The results show that females express different mate preferences in different choice situations. When a single female from a bottlenecked population was allowed to select one of two different males (female choice), she significantly preferred the ancestral male (Fig 5a; XX vs. XY : $z = 4.99$, $P < 0.0001$, $n = 95$; YY vs. YX : $z = 4.16$, $P < 0.0001$, $n = 95$), while derived females in choice situations where several females and males were present (multiple choice), showed no preference (XX vs. XY : $z = 0.83$, $P = 0.41$, $n = 58$; YY vs. YX : $z = 3.87$, $P = 0.0001$, $n = 58$). "Male choice" was not tested due to low sample size ($n=2$ and 4). The fact that derived females mated as ancestral females in female but not in multiple choice tests, together with the fact that the degree of discrimination was about the same in derived and ancestral females, implies that the derived females have retained their ancestral mate preferences but become more willing to mate with a non-preferred male when all the preferred males are unavailable to them. Hence the experimental treatment of derived populations has not

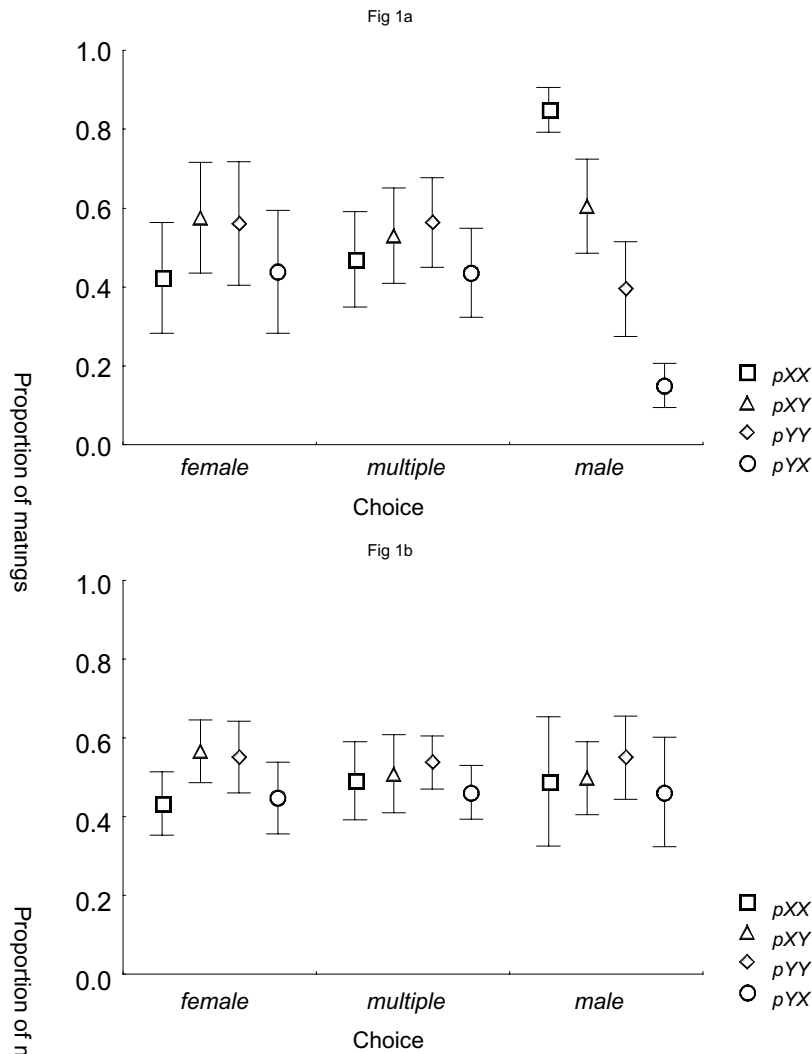


Figure 5. Proportion of matings of different types in different choice situation in a) peripatric experiments and b) vicariance experiments. Means and standard deviations are shown. X denotes the derived population and Y the ancestral, with females given first. The proportions are relative to the available mating types for females in female and multiple choice, and for males in male choice.

affected the actual preferences of females but only lowered their acceptance threshold for less attractive mates.

Kaneshiro's model has only been considered to apply to bottlenecked and unselected populations (Kaneshiro, 1976, 1980, 1983, 1989; Giddings & Templeton, 1983). However, we found the same kind of mating asymmetries in our compilation of unbottlenecked, vicariance experiments

(Fig. 5b). Furthermore, when the same tests as above were performed with only unselected lines the results did not change to any significant degree. This strongly suggests that mating asymmetries can arise, with or without selection, as a result of other than population bottlenecks, and constitute a more general phenomenon occurring when derived and ancestral populations meet.

So then, what do the derived populations have in common that makes their females prefer ancestral males? All derived populations, not only bottlenecked ones, have been kept at a lowered population size. In selected populations the selection itself lowers the effective population size, and also in vicariance-drift lines (Mooers et al., 1999), where neither selection nor bottlenecking had taken place, the populations had been kept at a much lower size than the ancestral population. It appears to be the lowered population size and the ensuing inbreeding that are the key factors generating the observed mating asymmetries, rather than the occurrence of true bottlenecks.

Since females in derived populations tend not to change their original preference, the mechanism suggested in Kaneshiro's speciation model is unlikely to reproductively isolate small daughter populations from their ancestor. However, two derived populations in secondary contact could very well be isolated from each other, because the ancestral male phenotype preferred by females from both derived populations will be present in neither. Hence, the Kaneshiro model remains a candidate for allopatric speciation on condition that it is limited to speciation between daughter populations.

A development of the Kaneshiro model (Paper III)

With the Kaneshiro model as a basic framework a new model is proposed to explain the results of Paper I and Paper II. It suggests that even minor reductions in population size and/or changes in selection pressures, which probably occur frequently during colonisation events, suffice for derived males to become less attractive and therefore discriminated against by all females that are given a choice. Furthermore, emigrating males probably are not a random sample of the ancestral population. On the contrary, it is plausible to assume that it is the least sexually successful males—carrying the least attractive traits—that have the most to gain from colonising new, less competitive habitats. In our model, as opposed to the Kaneshiro model, derived females lower their absolute thresholds for mate acceptance but continue to prefer ancestral males when given a choice. With retained

ancestral mate preferences, reproductive isolation between a mother and a daughter population—leading to speciation in the Kaneshiro model—will be slow to evolve. It is much more plausible that two derived populations become reproductively isolated from each other, not only because the preferred ancestral male phenotype will be present in neither population but because it is unlikely that parallel selection will evolve identical new male traits in both populations (but see Paper IV). Hence, this model is a strong candidate for the evolution of reproductive barriers between derived populations. Its candidacy is supported by the fact that derived populations in laboratory allopatric experiments demonstrate positive assortative mating when tested against each other but not when tested against an ancestral population (Paper I).

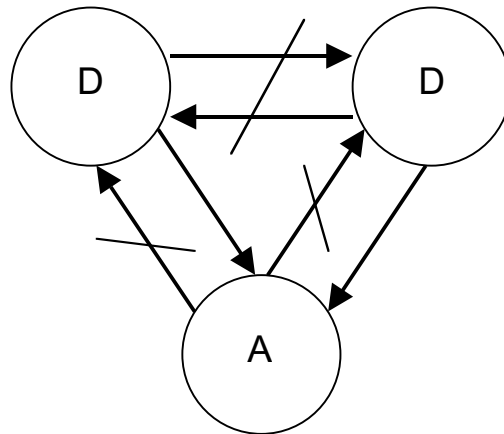


Figure 6. Mating asymmetries predicted by the model; arrows indicate absolute female preferences under free choice. The upper circles represent sister populations (D) derived from the same ancestral population (A).

This model offers a new look on post-glacial range expansions as a speciating mechanism. In a rapid re-colonisation of the north males may have lost attractive ancestral traits and females would have been selected to lower their acceptance threshold and to mate with less attractive males, since these were the only option available. The expansion flush would then act as a respite from stabilising selection on ancestral male phenotypes, allowing the development of new male traits, which could be subjected to sexual selection and become fixed, just as the Kaneshiro model predicts (Kaneshiro 1989). In the post-glacial north, sister populations might have come into secondary contact but would not interbreed for two reasons: i) neither population had retained the ancestral male phenotype and the

populations had lost different male secondary sexual traits (Mayr, 1942) (e.g. components of courtship behaviour or ornamentation) and females had only lowered their acceptance threshold for their own kind of male, or ii) the mating preferences of the different populations had by then diverged. However, the ancestral mating preferences would not necessarily have been lost before the short warm period was over, meaning that during the next ice age the derived and the ancestral populations might have collapsed into a single southern taxon again.

It might seem somewhat speculative to relate a model based mostly on mating asymmetry data from laboratory stocks of Hawaiian fruit flies to post-glacial expansions, but nevertheless the model offers some testable predictions. For example, sister populations would be more likely isolated from each other than from their ancestral population. This is a typical pattern of reproductive character displacement, such as one would expect after a history of reinforcement (Howard, 1993) or by isolation by distance in a species ring (Mayr, 1942; Wake 1989), but mating asymmetries as those depicted in fig. 6 are neither accommodated by ring speciation, nor by the reinforcement theory.

Radiation of yellow wagtails (Paper IV)

Many Palaearctic and Nearctic species form patchworks of distinct geographic variants. In recent years, several attempts have been made to explain their origin in the light of the major climatic changes caused by the cyclic glaciations of the Pleistocene epoch (Hewitt, 2000). During the peak of the last ice age ancestors of modern populations in the Holarctic must have taken refuge in the south. Populations in separate regions could then have evolved in isolation for many tens of thousands of years. However, some recent examples of speciation show that extensive phenotypic or behavioural differentiation can evolve with very little genetic change (eg. East African cichlids: Meyer, 1993; Seehausen & van Alphen, 1998 and Bowerbirds: Uy & Borgia, 2000). Typical of these radiations is that they involve dramatic differentiations of secondary sexual traits while leaving non-sexual morphology virtually untouched.

Despite being the focus of many decades of intensive interest and research, the Yellow Wagtail provides today one of the best-known examples of complex and unresolved intra-species relationships. The fact that its non-overlapping distributions (Fig. 1) are typical of young taxa (Barraclough & Vogler, 2000) suggests that the complex is young, and its northerly distribution implies that its composition has been largely affected by the

Quaternary ice ages. But glacially induced diversification by population subdivision among southern refugia may not reveal the whole story. There is a very large number of subspecies compared to the number of plausible refugia, and the fact that the subspecies are almost exclusively differentiated in a few male plumage traits is not expected from long-term geographical isolation. In contrast to plumage, song appears to be extremely simple in all yellow wagtails subspecies. This discrepancy between secondary sexual traits might hold a clue to the formation of the species complex.

Many related species to the yellow wagtails have quite complex songs. It is therefore likely that the ancestor of today's yellow wagtails also had more complex song. It has been shown in several animal groups, including birds, that the loss of sexually selected male traits is a widespread evolutionary trend (review in Wiens, 2001). A number of explanations for this counterintuitive phenomenon have been proposed, but the Kaneshiro model (1989) is the only model for speciation by sexual selection that makes explicit assumptions about loss of secondary sexual traits. A central assumption of this model is that new traits can become favoured because some other trait under sexual selection in an ancestor has been lost. For yellow wagtails, the loss of a once variable song might have been replaced by elaborate plumage coloration. This study attempted to trace the mechanism behind the differentiation of yellow wagtails, by relating molecular data in the form of population statistics and molecular phylogeny (mitochondrial DNA and a Z-chromosome intron sequence) to secondary sex trait variation.

Gene trees of *Motacilla flava* showed two major patterns. First, the species was found to be polyphyletic with a deep split, forming two well-defined clades with different most recent ancestors, i.e. a Western Palaearctic and an Eastern Palaearctic clade (Fig. 7). Second, it was not possible to get any strong resolution among the subspecies. For mtDNA, the average genetic distances suggest that the species complex is very young, especially the western clade (within the Western clade: 0.44 % (range: 0.089 – 1.02%; within the Eastern clade: 1.40%, range: 0.019 – 2.00%, Maximum likelihood distances between subspecies).

Genetic structure of four subspecies more extensively sampled indicated that a population bottleneck has occurred during expansion from Mediterranean habitats. A pair-wise sequence comparison approach (Rogers, 1995) showed that the southern as opposed to the northern subspecies pair did not conform to a model of sudden

Bootstrap

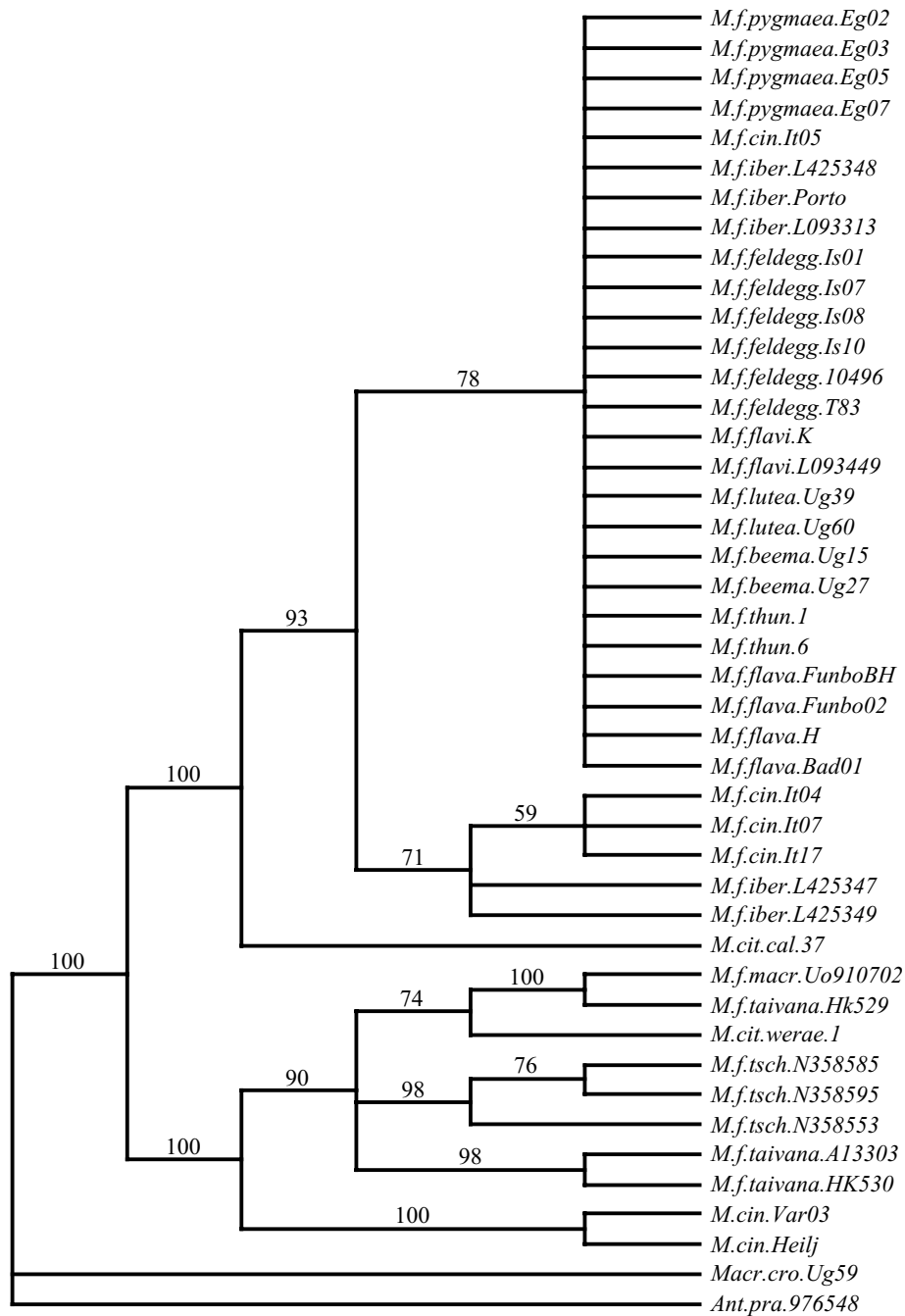


Figure 7. Maximum parsimony tree based on mtDNA with bootstrap support values (1000 replicates) shown above the nodes.

population expansion ($P = 0.03, 0.024$). Haplotype diversity decreased with latitude (Table 1 and Fig 8) Genetic structuring among subspecies was evident, but very little haplotype variation could be attributed to subspecies differences within northern and southern parts of Europe ($F_{sc} = 0.05, P < 0.05$). This does not conform to expectations from long-term differentiation in glacial refugia.

Mean number of different elements in a single syllable of song also decreased with latitude.(Fig 9; Kruskal–Wallis: $H = 16.46, P = 0.0009$).

Table 1. Subspecies, collection localities and pair-wise differences in haplotype diversity.

Subspecies	Locality	Approx. latitude	Comparison	Difference	P
1. <i>M. f. pygmaea</i>	N. Egypt	30°	1–2	0.047	0.61
2. <i>M. f. cinereocapilla</i>	N. Italy	45°	1–3	0.228	0.001
3. <i>M. f. flava</i>	S. Sweden	58–60°	1–4	0.271	0.0002
4. <i>M. f. thunbergi</i>	N. Fennoscandia	65–70°	2–3	0.181	0.049
			2–4	0.224	0.0074
			3–4	0.043	0.92

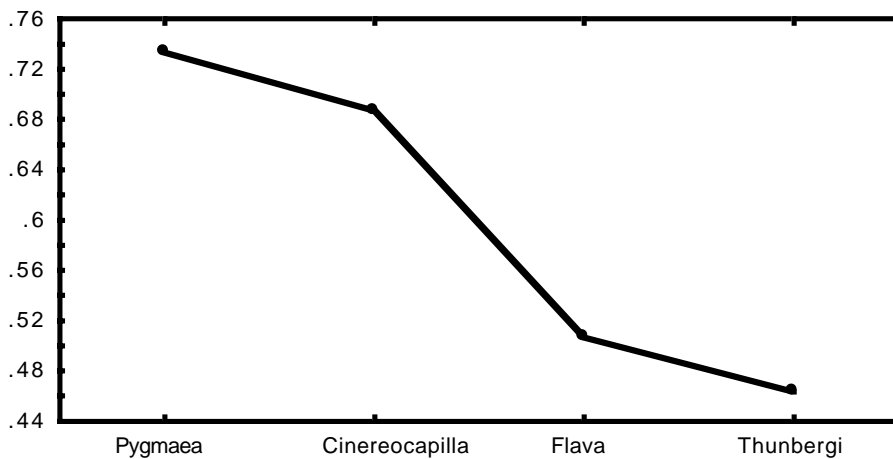
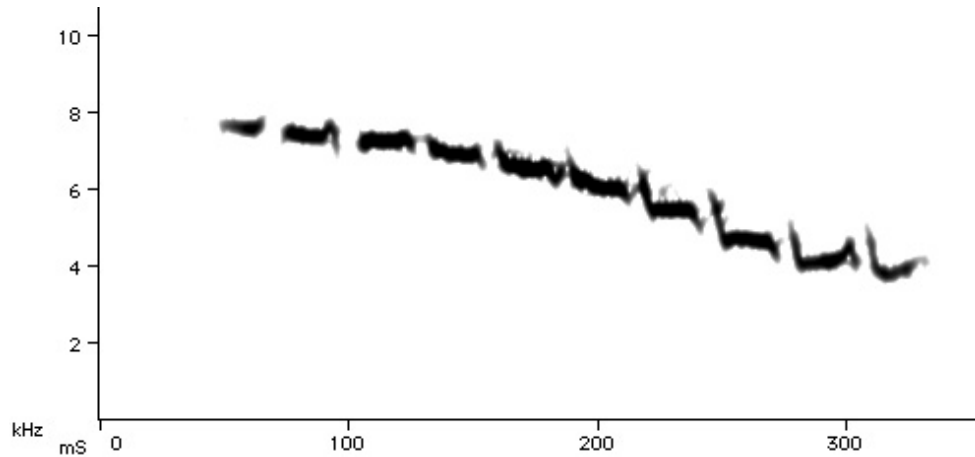


Figure 8. Haplotype diversity in four populations of different yellow wagtail subspecies.

M. f. thunbergi: Orsa, Dalarna, Sweden



M. f. pygmaea: Dhasur, S. of Giza, Egypt

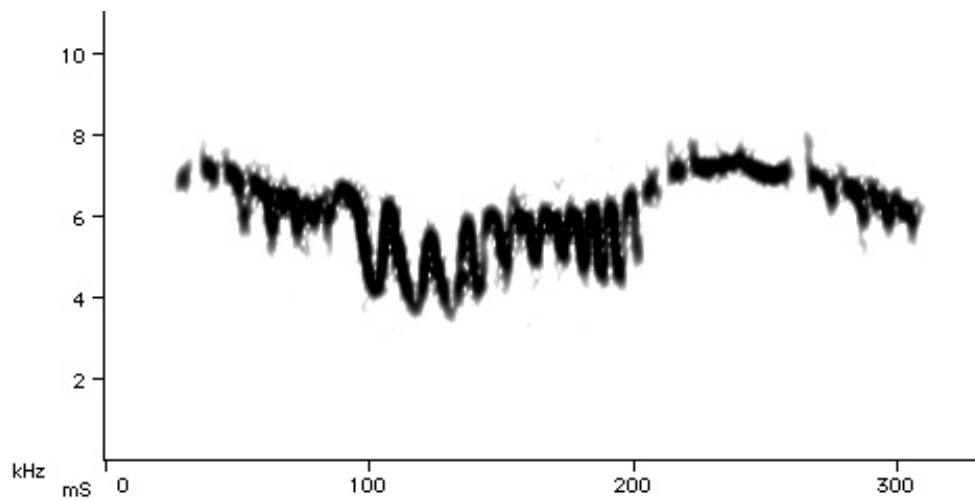


Figure 9a. Sonagrams showing one syllable of song.

This study hence presents correlative evidence that Kaneshiro's (1989) speciation by sexual selection process might have formed the Yellow Wagtail complex. The Kaneshiro model assumes that new traits become favoured because some other trait under sexual selection in an ancestor has been lost. Although inaudible to humans, the northern yellow wagtail subspecies definitely seem to have had their vocal repertoire reduced in connection with a population bottleneck.

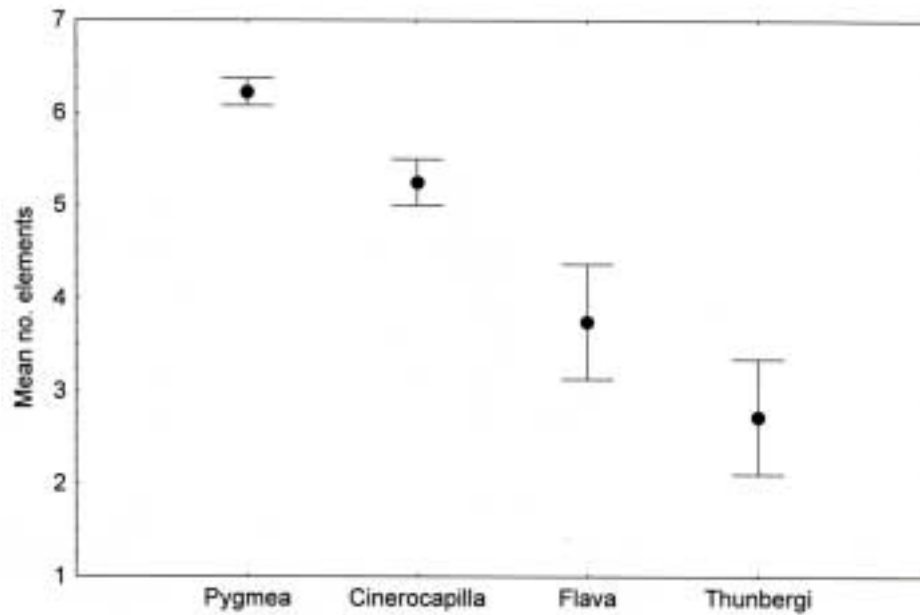


Figure 9b. Mean number of elements in a single syllable of song in four yellow wagtail subspecies. Whiskers represent standard errors.

Sex trait evolution in wagtails (Paper V)

Speciation usually refers to the evolution of reproductive isolation (Mayr, 1942; Turelli *et al.*, 2001), in agreement with the “biological” species concept (Mayr, 1942). Under this concept, the taxonomic rank of natural groups is judged on the basis of their degree of intergradation in areas of contact (sympatry or parapatry) and, in cases of geographical isolation (allopatry), on the degree of differentiation that is assumed to serve as barriers to reproduction in contact zones. Accordingly, one has to assume that reproductive isolation and morphological differentiation always show correlated variation. However, reproductive isolation can evolve without concomitant morphological differentiation, as in e.g. “sibling species”. Conversely, divergence in morphological traits does not necessarily lead to intrinsic reproductive isolation, as is exemplified by numerous taxa classified as subspecies of polytypic “biological” species.

The Wagtails (genus *Motacilla*) are suitable for the study of speciation, because they form a phenotypically very diverse group, while apparently being of recent origin. This is suggested by the fact that all wagtails are fairly similar in size and structure, in spite of their plumages being highly

divergent. To obtain an as accurate estimate as possible of the evolution of this genus molecular phylogenies were constructed by sampling all least-inclusive taxa, regardless of their taxonomic rank. The usual procedure in avian systematics is to sample only those taxa that qualify as biological species (see above). With that method, there is a severe risk of underestimating divergence rates. One might come to false conclusions on the relative importance of different speciation processes due to the fact that only old taxa are sampled. There is a possibility that sampling at the species level will bias evidence in favour of geographical modes of speciation.

Trees based on mitochondrial and nuclear sequences were incongruent with each other and with current systematics. The most striking dissimilarities between the trees and deviations from traditional, non-cladistic classifications based on morphological data are shown in Table 2.

Table 2. Main discrepancies between trees based on mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) and between either of these and current taxonomy (Mayr & Greenway, 1960; Alström *et al.* in press). Agreement refers to general agreement, since no phylogenetic analysis has been carried out previously.

	mtDNA–current classification	nDNA–current classification
<i>M. aguimp vidua</i>	Paraphyletic with respect to other “black-and-white” wagtails (clade B).	Agreement.
<i>M. grandis</i>	Agreement.	Agreement, except for one individual (no. 2).
<i>M. c. cinerea</i>	Sister to eastern <i>M. flava</i> and <i>M. c. citreola</i> (clade C) and Asian “black-and-white” wagtails (clade B).	Sister to eastern <i>M. flava</i> (though support weak) and position uncertain
<i>M. flava</i> <i>ssp.</i>	Non-monophyly (western and eastern taxa form separate clades).	Non-monophyly (western and eastern taxa form separate clades).
<i>M. citreola</i> <i>ssp.</i>	Non-monophyly (<i>citreola</i> inside eastern <i>M. flava</i> clade, <i>calcarata</i> within western <i>M. flava</i> clade).	Agreement.
<i>M. alba</i> <i>ssp.</i>	Agreement, except for one individual of <i>subpersonata</i> (no. 2).	Agreement.

The incongruence between the two trees and between either of these and morphology-based classifications (Table 2) demonstrates the necessity to base phylogenetic estimates on multiple independent data sets. Only when

such data sets result in congruent patterns is it possible to obtain a well supported organismal phylogeny. However, nearly all avian molecular phylogenies are based only on mitochondrial sequences.

The divergence in mtDNA is extremely low between the different subspecies of *M. alba* and western *M. flava*, considerably lower than between most currently recognised species of wagtail (Fig. 10). This result is in striking contrast to the strong plumage differentiation among the conspecific taxa. In fact, the authors are not aware of any other group of birds, except for some closely related species of ducks in the genus *Anas* (Johnson & Sorenson, 1999), that are so heterogeneous in male breeding plumage while being comparatively homogeneous in mtDNA.

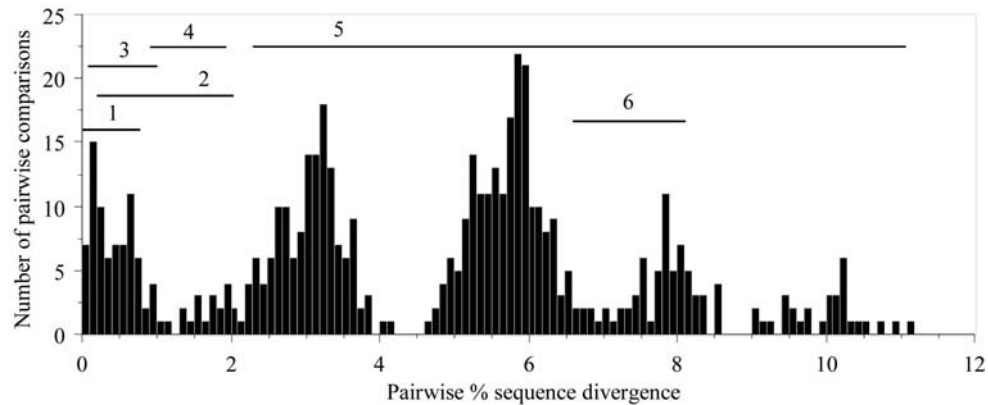


Figure 10. Distribution of pairwise mtDNA sequence divergence among wagtails. (1) between taxa in the *M. alba* complex, (2) between taxa in the eastern *M. flava* complex, (3) between taxa in the western *M. flava* complex, (4) *M. citreola* – eastern/western taxa in the *M. flava* complex, (5) between reproductively isolated species except *M. citreola*–*M. flava* complexes, (6) *M. clara torrentium*–*M. capensis capensis*–*M. flaviventris*. The small divergence between *M. citreola* and the *M. flava* complexes may be due to introgression (cf. Fig. 11).

The extremely short internal and terminal branches within especially the *M. alba* and western *M. flava* complexes (Fig. 11) suggest a recent, explosive radiation of these. However, recent bursts of speciation are not corroborated by a comparison of empirical data with a null distribution according to a random birth-death model (Fig 12: Wollenberg *et al.*, 1996; $n=33$, $D=0.35$, $p<0.01$), indicating an early rather than a late pulse of speciation. However, the Wollenberg *et al.* (1996)

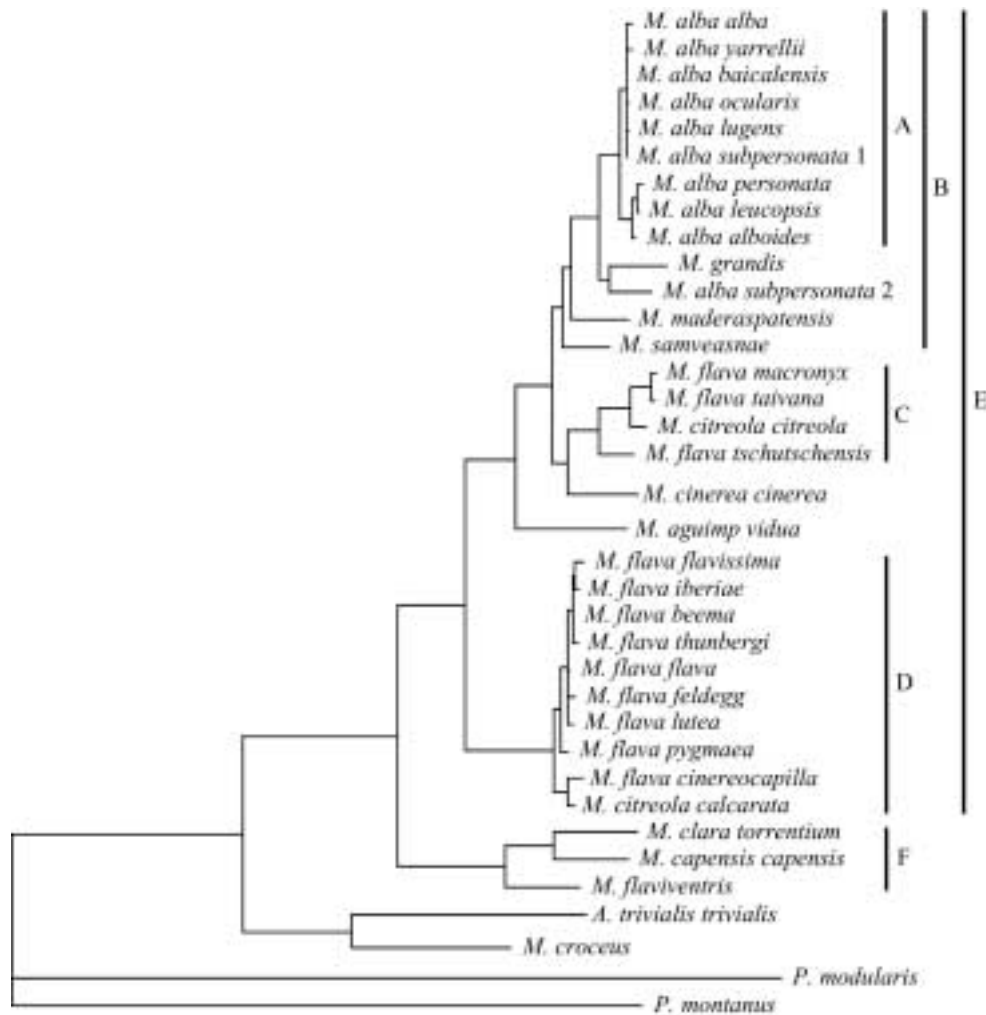


Figure 11. Maximum likelihood tree based on mitochondrial sequences.

test does not take into account that the radiation of wagtails is non-random within the phylogeny; half of the diversity stems from only two nodes, representing the ancestors of the *M. alba* and the western *M. flava* complexes, respectively. The presumably very recent origin of these two groups coupled with their extremely strong plumage differentiation is virtually unparalleled in the avian world. Which processes may be responsible for this extraordinary differentiation?

Some observations suggest that the phenotypic divergence among the wagtails is not due simply to drift in geographical isolation. (1) Morphological differences between taxa are almost entirely related to

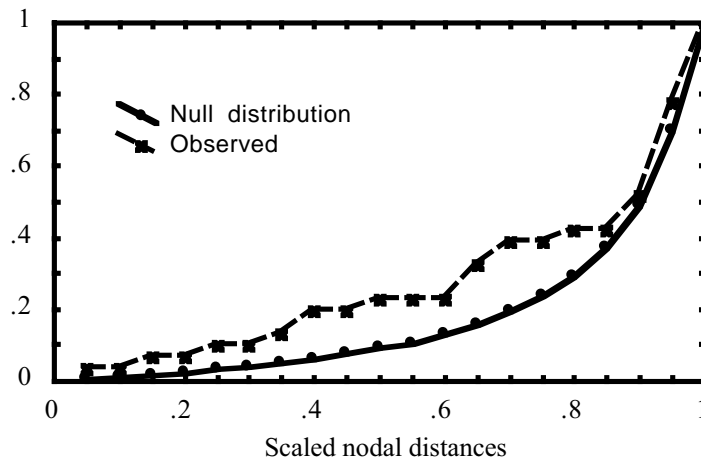


Figure 12. Cumulative distributions of base to node distances.

plumage, in particular male breeding plumage. Drift is unlikely to affect sexual traits more than other traits. (2) Many taxa that are well differentiated in plumage differ very slightly genetically (e.g. the taxa in the *M. alba* and western *M. flava* groups), while some distant relatives are closely similar in plumage (notably *M. aguimp*–*M. samveasnae* and some of the taxa in the two *M. flava* complexes). The opposite pattern would be expected from drift. (3) Although geographically isolated distantly related taxa generally differ in a larger number of plumage traits than neighbouring closely related taxa, the differences are often less conspicuous in the former than in the latter group.

There are indications that chance factors may have been important in the evolution of wagtails; many of the northern wagtail taxa that are very widely distributed and have large populations show very little genetic within-taxon variation. Similar patterns found in other avian groups have been interpreted as evidence for peripatric speciation (references in Grant & Grant, 1997). However, classical peripatric speciation theory (Mayr, 1954; Carson, 1975; Templeton, 1980) makes no explicit assumption that secondary sexual traits would be favoured over other morphological characters. Therefore, it does not seem to explain the evolution of the wagtails.

Although we lack direct evidence of sexual selection in wagtails, this seems to be the most plausible explanation for the evolution of plumage differences between the sexes as well as between most of the taxa. The fact

that clade E (Fig 11), which includes only sexually dichromatic taxa is six times larger than its sister clade (F), in which all taxa are sexually monochromatic, strongly suggests that sexual selection on plumage has played a role in the diversification process. Furthermore, unlike the taxa in clade F, those in clade E display sexual variation in size, seasonal plumage variation and age-related differences, indicating greater potential for sexual selection in clade E than in clade F.

Observations do not comply with a differentiation by reinforcement. (1) In general, parapatric taxa hybridise regularly, producing broad hybrid zones in some areas (review in Alström *et al.*, in press). This suggests that postzygotic isolation, which is the driving force behind reinforcement, is weak or non-existent in these taxa. (2) The traits that differ between taxa that are in contact are homogeneous throughout the respective ranges of these taxa. Reinforcement should lead to exaggeration of differences in and close to the contact zone. (3) The time scale for the divergence of the *M. alba* and western *M. flava* (Fig. 10) is probably too short to accommodate the three stage process required for reinforcement: morphological and genetic differentiation in isolation, range expansion followed by exaggeration of differences in contact zones, and subsequent spread of the resulting phenotypes throughout the respective range.

Instead, this study suggests that sexual selection on male plumage traits was triggered by founder events, in agreement with the peripatric speciation model of Kaneshiro (1989). Apart from plumage, song is the only trait that varies considerably between taxa. While plumage diversity is greater in temperate than in subtropical and tropical regions, the opposite is true for song. The four Afrotropical taxa and *M. maderaspatensis*, *M. samveasnae*, *M. grandis* and *M. cinerea* all have complex, varied songs, while the *M. alba*, *M. flava* and *M. citreola* groups have extremely simple songs. Ignoring the rather northerly-distributed *M. cinerea* and *M. grandis*, the complexity in song seems to correlate inversely with latitude. However, the correlation with terminal branch lengths and relative positions in the tree (Fig 11) is even better. Every taxon with a simple song is positioned on a short terminal branch, and basal taxa have more complex songs than more derived taxa. Song is to a great extent culturally transmitted in songbirds (oscines). It has been shown that absence of tutors during critical periods in the learning process can lead to poor development of song, probably to the exclusive expression of the genetically inherited template (Kroodsma, 1982; several references in Kroodsma & Miller, 1996). In the wagtails, song complexity could have been lost in a similar fashion during bottleneck events in connection with northward expansions. Although there is no

direct evidence that loss of song variation has led to diversification in plumage, the indirect support is rather strong.

CONCLUSIONS

In the first chapters of this thesis I review the published record of laboratory experiments of allopatric speciation. I demonstrate that few of the factors traditionally believed to increase the likelihood of allopatric speciation have been particularly relevant. However, the method of testing reproductive isolation seems to be important. Laboratory sister populations tend to demonstrate reproductive isolation against each other, but not against the ancestral population. This discrepancy seems to be an effect of asymmetric mate preferences for ancestral males. In agreement with the basic framework of Kaneshiro's speciation by sexual selection model, I propose that the responsible mechanism is that experimentally derived males lose attractive traits and that females from the same population are selected to lower their mate acceptance thresholds. This relaxes sexual selection on ancestral phenotypes and allows males to evolve novel secondary sexual traits. In temperate regions, geographical isolation in refugia during glaciations has been shown to be of major importance in promoting speciation. However, some groups of animals, as for example some clades in the wagtail genus discussed in this thesis, seem to have radiated not as a consequence of isolation but from changes that occurred during post-glacial northward range expansion. These expansions may have triggered shifts in sexual selection leading to rapid evolution of new secondary sexual traits. Wagtail males of leading edge populations seem to have lost song complexity and females may have favoured aberrant plumage phenotypes instead. It has been shown in several animal groups, including birds, that the loss of sexually selected male traits is a widespread evolutionary trend. A multitude of explanations have been proposed, but surprisingly the Kaneshiro model is not one of them, despite being the only model of speciation by sexual selection that makes explicit assumptions about loss of secondary sexual traits. The mating asymmetries believed to result from the Kaneshiro process are strongly supported by evidence from laboratory populations. It seems possible that this phenomenon has been of significant importance to the diversification of many different groups of animals, in particular taxa that have been subject to drastic changes in distribution and populations size over time, such as many northern and island species.

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