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Genetic patterns of Black-tailed Godwit populations and their implications for conservation

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CHAPTER

7

General Discussion

General discussion

In this thesis Black-tailed Godwits (*Limosa limosa limosa*) from a range of breeding areas were genetically compared at a regional (in southwestern Fryslân), national (among most prominent breeding areas in the Netherlands), and global scale (among breeding areas from across the entire breeding range of the species) to increase our understanding of the effects of habitat fragmentation and habitat quality on this species. In The Netherlands, the Black-tailed Godwit has evolved into an agriculture-following species. Unfortunately, increasing intensification of agricultural practices, such as early mowing of grasslands, conversion of grassland to cornfields, drainage of the meadows and high cattle densities have influenced habitat quality and quantity negatively. This has resulted in stark decreases in recruitment (Roodbergen *et al.* 2012, Schekkerman *et al.* 2008;2009).

With the loss of settlers and the loss of good breeding habitats, over the last decades Black-tailed Godwit breeding areas have become fragmented over much of the breeding range, yet little is still known about the influence of such habitat fragmentation. With the work in this thesis an attempt has been made to provide an insight in the effects of habitat fragmentation on the population dynamics of Black-tailed Godwit populations at different spatial scales and the long term.

1. Can eggshell membranes be used as a reliable DNA source in genetic research?

To conduct genetic research, DNA samples are needed. The most widely used DNA source for this kind of research has been blood samples. Blood samples usually provide clean DNA of high quality. However, birds of which blood samples need to be obtained, have to be captured which causes a certain amount of stress. Additionally, capturing enough birds is time consuming and requires specialists. Consequently, sampling blood for genetic studies could limit the geographical scale on which genetic studies can be done. A faster and less invasive method of DNA sampling would allow large-scale assessments of genetic diversity and genetic differentiation. Fragments of egg shell are often left in the nest after hatching, and are easily collected by volunteers. The inner membrane of egg shells grows veins with blood of the fetus during the later stages of development, which could provide the DNA needed for genetic research. However, egg shell membranes are prone to contamination with bacteria and or viruses, which could potentially degenerate the genetic information that can be obtained. Furthermore, cross contamination from other egg-shell membranes in the same nest could prove to be problematic. The aim here was to investigate the usefulness of eggshell membranes as a DNA source for genetic research of egg laying species like birds and reptiles, by addressing eggshell membrane DNA quality, degeneration and cross-contamination. To this end a comparison was made between DNA from eggshell membranes with blood-derived DNA samples from the same individuals. No degeneration, cross contamination or deteriorated DNA quality were detected. Even in total genotypic comparison of eggshell membrane DNA and blood sample DNA, using 11 microsatellite loci, neither degeneration nor cross-contamination was apparent. This research clearly illustrates that eggshell membranes can be used for population genetic research. Therefore, apart from the blood samples already collected in other studies, that we had excess to, egg shell membranes were also collected as a DNA source in the rest of this thesis. Through this method a higher geographical sampling coverage of the Black-tailed Godwit breeding areas was possible.

2. *What are the genetic differences between breeding populations on intensively managed agricultural grassland and extensively managed agricultural grassland?*

In a previous study it was suggested that intronic variation in the CHD1-Z gene (CHD1-Z*) of Black-tailed Godwits (*Limosa limosa limosa*) breeding in southwest Friesland, The Netherlands, was under selection or linked to another gene that was under selection (Schroeder *et al.* 2010). Individuals with the CHD1-Z* allele were found in high quality habitat in a higher frequency than individuals without the CHD1-Z* allele, which indicated genetic population structure correlated with habitat quality on a local scale. Furthermore, it was shown that the presence of CHD1-Z* correlated with fitness components. Here we re-examine these interesting correlations using a much expanded dataset (on 2088 birds from 2004-2010 rather than 284 birds from 2004-2007). The presence of the CHD1-Z* allele showed a tendency in adult females to lay their clutches earlier. Additionally, the occurrence of the CHD1-Z* allele resulted in chick body mass and return rates being higher. Chicks with the Z* allele that had hatched early in the breeding season were heavier at birth compared to chicks without the Z* allele and chicks with the Z* allele that had hatched late. However, individuals carrying the CDH1-Z* allele did not occur more on extensive agriculturally managed land compared to intensive agriculturally managed land. This research indicated that the CHD1-Z* allele correlated with fitness measurements in the chicks and probably in adult females, suggesting that there is some form of positive selection working on Black-tailed Godwit populations in southwestern Fryslân: female adults with the CHD1-Z* allele had a trend towards laying earlier nests, followed by possible CHD1-Z* chicks which had a higher return rate in general and body mass if hatching occurred early in the season (which indirectly reflects early laid nests). Such a mechanism can be explained by the following line of reasoning. If females lay earlier clutches, these nests would have a higher chance to survive mowing practices on agricultural land (which generally take place during the second half of May) (Kleijn *et al.* 2010).

One recent study showed that in general Black-tailed Godwits did not adjust their laying date to earlier dates (Musters *et al.* 2010). This could either indicate that there is no advantage for Black-tailed Godwits to lay their clutches early, or that they are somehow unable to advance their breeding schedule. However, other studies showed that Black-tailed Godwits do have higher survival if they lay their clutches earlier (Kleijn *et al.* 2010; Schroeder et al. 2012). If females have the ability to lay early clutches it seems these females (carrying the CHD1-Z* allele) would simultaneously produce chicks, if such a chick also carried the CHD1-Z* allele, with higher body mass and return rates. Chicks with high body mass would have a higher survival during the most critical stages of development, which is suggested to lead to higher survival in general (Arnold *et al.* 2006, Schekkerman *et al.* 2008; 2009, Schroeder *et al.* 2012). Musters *et al.* (2010) suggested that the reason for not shifting their lay date, might be that the peak of adult Black-tailed Godwit food (earthworms) abundance, did not change to earlier dates over time either. This would indeed deprive Black-tailed Godwit females of adjusting their lay date to earlier dates. So, while in general Black-tailed Godwits seem unable to shift breeding to an earlier date, it seems that indeed it gives a selective advantage to breed earlier. These results indicate the importance of early-laid clutches and chick body mass for chick recruitment and thus increased survival, which has been demonstrated in a growing number studies over the last decade (Kentie *et al.* 2013, Kleijn *et al.* 2010, Schekkerman *et al.* 2007;2008;2009, Schroeder *et al.* 2012)

No direct correlations could be found between the presence of CHD1-Z* and habitat quality. In other words no genetic differences could be observed between breeding populations on intensive agricultural grassland and extensive agricultural grassland. On the other hand it was shown that return rates of chicks that had hatched on extensively managed agricultural land were higher than chicks that had hatched on intensively managed agricultural land. Chick recruitment (from egg to adult breeding bird), which can be loosely translated to chick return rates, is shown to be the most important determinant of current Black-tailed Godwit population trends (Schekkerman *et al.* 2008, Roodbergen *et al.* 2008, Kentie *et al.* 2013). The finding presented here, in regards to habitat quality, highlights the importance of extensive agricultural grassland management for Black-tailed Godwit survival, which is supported by several recent studies (Groen *et al.* 2012, Kentie *et al.* 2013, Schekkerman *et al.* 2007;2008;2009). Moreover, recent research postulates that focusing habitat restoration efforts on well connected, large areas with a minimum size of 100 ha and that are still rich in Black-tailed Godwit breeding numbers should safeguard the Black-tailed Godwit population in the Netherlands on the long term (Teunissen *et al.* 2012).

3. What are the genetic differences between geographically isolated breeding populations in The Netherlands?

Besides selection and decreasing habitat quality other processes can disrupt viability of natural populations. Habitat fragmentation could in theory severely impair gene flow between neighboring breeding areas, which would in turn increase the effects of inbreeding and genetic drift in these fragmented breeding populations (Frankham 2010). However, the extent of this effect is highly dependent on the effective population sizes of these populations, the dispersal capabilities of the species and their breeding site faithfulness. The Black-tailed Godwit is a migratory bird flying thousands of kilometers from breeding area to winter area and back. As already mentioned in the introduction, Groen (1993) showed 90% of the adult breeding Black-tailed Godwits returning within 700 m of the previous nest site. Natal philopatry was demonstrated as well with 75% of the birds returning within 18 km of their previous hatching site (Groen 1993). These findings were further supported by Kruk et al. (1998) who showed first time breeders dispersed beyond 50 km in regards to their previous hatching site. However, since it is largely unknown what effective population sizes are and if gene flow occurs on even larger spatial scales, it is still unclear if and if so, at what geographical level habitat fragmentation influences Black-tailed Godwit breeding populations. Therefore we investigated genetic diversity at and genetic differentiation and gene flow between different Black-tailed Godwit breeding locations within the Netherlands on the basis of 12 microsatellites. The distance between breeding areas ranged from 7 to 135 kilometers, which was far beyond dispersal distances reported in demographic studies up till now (Groen 1993, Kentie *et al.* 2011, Kruk *et al.* 1998, Van den Brink *et al.* 2008). No genetic differences, with either F_{ST} or D analysis, could be detected and gene flow estimates were larger than "one migrant per generation" between breeding areas. These findings indicate that the dispersal abilities of the Black-tailed Godwit far exceed the 18 km maximum reported in demographic studies and possibly stretch beyond 135 kilometers. If this is indeed the case, most breeding areas in the Netherlands are still connected through dispersal of one or more individuals per generation.

However, incomplete lineage sorting could also explain the lack of genetic differences found between Black-tailed Godwit breeding areas. Lineage sorting in turn depends on effective population size and time since divergence took place (Zink and Barrowclough 2008). Since the geographical fragmentation currently observed between Black-tailed Godwit breeding areas in the Netherlands is believed to have started roughly 50 years ago, it is likely that lineage sorting is not complete for theoretical splits currently present between Godwit breeding areas. Effective population sizes can also influence lineage sorting. The larger the effective population in two geographically isolated populations the longer it will take for both lineages to sort i.e., become genetically distinct from each other (Frankham 2010, Zink and Barrowclough 2008). Usually, effective population size is smaller than the estimated population size. So, if the number of breeding pairs at the geographically isolated breeding areas in the Netherlands could be considered real population sizes, effective population sizes in these isolated areas would be small and thus lineage sorting would be fast. However, breeding areas that seem geographically isolated are not necessarily the relevant populations. For instance when a breeding area is made up out of three geographically isolated patches, but these three areas are still linked together through gene flow, then effective population size in the breeding area could actually be quite large, while estimated populations would indicate three small populations. Therefore, lineages might not have had enough time to sort due to effective population sizes being large at the different sampled Black-tailed Godwit breeding areas. Nevertheless, it is clear that fragmentation events have not impacted Black-tailed Godwit breeding areas in the Netherlands (on a scale of 7 to 135 km) to such an extent that they can be shown through genetic analysis. This demonstrates the high dispersal capabilities of the Black-tailed Godwit.

There is, however, another factor that might have been influencing the genetic patterns, or rather the lack thereof, observed. Effective population size at neutral loci, like the microsatellites used here, are frequently four times that of the mitochondrial locus, because of recombination and mitochondrial DNA being only maternal inherited in most cases (Frankham 2010). Therefore, mitochondrial DNA might detect recent splits for which nuclear loci did not have time to sort (Zink and Barrowclough 2008). So, in theory mitochondrial DNA might have picked up genetic differences between Dutch Black-tailed Godwit breeding areas. The scale of the study area, the effective population sizes of the sampled areas, and DNA marker used in this study could thus have limited our ability to detect genetic differences. Therefore, global scale investigations of genetic structure were performed using mitochondrial DNA and nuclear DNA (see below).

4. What are the genetic differences between breeding populations on a global scale?

Breeding areas were sampled at different parts of the Black-tailed Godwits entire breeding range, including breeding areas of the three morphologically recognized supspecies (*L. l. limosa, L. l. islandica* and *L. l. melanuroides)*. Population genetic structure and phylogeographic patterns were analyzed between these breeding areas using three different parts (COI, HVR1 and HVR2) of the mitochondrial locus (mtDNA) next to the 12 microsatellite loci (nuDNA). Since mitochondrial DNA was used here, which should be able to pick up smaller genetic differences than microsatellites due to their faster lineage sorting, we included many samples of the Dutch Black-tailed Godwit breeding areas

(Zink and Barrowclough 2008). In previous studies mtDNA sequence data showed minimal genetic divergence between the three subspecies and an absence of substructuring within *L. l. limosa*. Here, the nuDNA data suggested slight genetic differentiation between *L. l. limosa* from Sweden and the Netherlands with other *L. l. limosa* sample locations showing genetic admixture between Dutch and Swedish genotypes, between *L. l. limosa* and *L. l. islandica*, but not between *L. l. limosa* and *L. l. melanuroides*. The mtDNA also demonstrated a split between *L. l. limosa* and *L. l. islandica* and showed two *L. l. limosa* haplotype clusters that were not geographically isolated. This result also included Dutch Black-tailed Godwit breeding areas that were re-examined using mtDNA. So, while different mtDNA haplotypes were found within the Netherlands they did not correspond to a geographical structuring of the Black-tailed Godwit breeding areas, again supporting the notion that Dutch Black-tailed Godwit breeding areas are still connected genetically. However, the mtDNA data did not seem to be completely consistent with the nuDNA pattern as mtDNA did support a split between *L. l. melanuroides* and *L. l. limosa*/*L. l. islandica.* And while the *L. l. limosa* haplotype groups showed more genetic detail than the genotype *L. l. limosa* clusters in the nuDNA, the general *L. l. limosa* pattern was similar in both markers, with the exception of the genetic pattern represented by Dutch *L. l. limosa* individuals. The discordance between nuDNA and mtDNA genetic structure, in regards to the split between *L. l. melanuroides* and *L. l. limosa/L. l. islandica*, might be explained by contamination of the DNA, homoplasy, small sample size, introgression, incomplete lineage sorting or a combination thereof (Ballard *et al.* 2002, Zarza *et al.* 2011, Zink and Barrowclough 2008).

Within *L.l. limosa*, mitochondrial COI sequences were 100% identical for 57 individuals from samples throughout the *L. l. limosa* distribution, which could indicate contamination issues. However, COI sequences were derived from different PCR batches, with samples from diverse sources including blood, eggshell and muscle tissue, from which DNA was extracted by different people and in different laboratory rooms. Moreover, most public sequences of *L. l. limosa* that were also added to our dataset, resulting from sequence runs performed in different labs around the world and by different research groups, also consisted of this most common haplotype. Additionally, the same DNA isolates were used in the microsatellite and mitochondrial HVR analysis. These analyses did not show identical DNA sequences or microsatellite genotypes in *L. l. limosa* for these individuals comparable to the COI structure for the most common haplotype. In every single PCR, fragment length run and sequence run, for every single marker, negative controls were added and analyzed to further exclude contamination issues. No contamination was found in any of the analyses performed. Therefore contamination issues can be ruled out.

Additional analysis indicated the presence of homoplasy within our dataset. Homoplasy occurs when microsatellite alleles have identical fragment lengths that are not identical by descent (Selkoe and Toonen 2006). Although the presence of homoplasy might cause underestimation of population genetic structure, simulation studies suggest that any bias thus introduced will be only slight and will generally have much less effect on estimates of population differentiation than migration or genetic drift (Estoup *et al.* 2002, Selkoe and Toonen 2006). Moreover, the less distant splits between *L. l. islandica* and *L. l. limosa* and the haplotype groups within *L. l. limosa* were displayed by the mtDNA. It is therefore unlikely that homoplasy issues had a conservative effect on the *L. l. melanuroides* signal only.

The most likely reason for the discordance in the *L. l. melanuroides* signal between nuDNA and mtDNA is probably small sample size. Additional microsatellite analysis with pruned sets of three randomly chosen samples per location indicated that microsatellite analysis, including STRUCTURE, are conservative with regards to assigning samples to a different groups. For instance, when the *islandica* group was limited to 3 individuals no clear signal became visible using STRUCTURE. Alternatively the different *L. l. melanuroides* signals found might be explained through a scenario of isolation of *L. l. melanuroides* from *L. l. limosa* in Beringia during the Last Glacial Maximum, possibly followed by recent introgression (visible in the nuDNA only). Combined COI results of our *L. l. melanuroides* samples and previously analysed *L. l. melanuroides* samples, with some samples from the same sample location, showed two groups, one demonstrating divergence from *L. l. limosa* and while the other group sorted very close to *L. l. limosa* (Elbourne 2011). These groups might constitute two disjunct *L. l. melanuroides* breeding colonies. An alternate hypothesis would be the presence of a slightly diverged *L. l. limosa* breeding population and a *L. l. melanuroides* breeding population, suggesting current overlap in *L. l. limosa* and *L. l. melanuroides* breeding sites.

With respect to the other genetic signals found, the nuDNA and mtDNA suggested relatively recent separation of *L. l. islandica* and *L. l. limosa*. Additional analysis indicated that during the Pleistocene separation of *L. l. islandica* from *L. l. limosa* occurred, followed by colonization of Iceland by the *L. l. islandica* during the Holocene. Within *L. l. limosa* founder events followed by population expansion that took place during the Holocene explained the genetic patterns in the mtDNA nicely. Incomplete lineage sorting regarding the theoretically existing and relatively recent splits in the *L. l. limosa* could explain why genetic structure found on the basis of nuDNA was less prominent than the structure found on the basis of mtDNA. Alternatively, the Dutch *L. l. limosa* population expansion that took place during the first half of the 20th century (Beintema *et al.* 1995, Haverschmidt 1963) may have caused introgression among Dutch *L. l. limosa* breeding locations. This could have resulted in the genetic homogenization of the Dutch *L. l. limosa* breeding population in the nuDNA. Moreover, it appeared that this recent *L. l. limosa* population expansion has resulted in introgression between Dutch *L. l. limosa* individuals and individuals from other *L. l. limosa* breeding locations as well. Some divergence between Sweden and other *L. l. limosa* sampling locations was shown by the nuDNA. While the Swedish *L. l. limosa* individuals did not share any mtDNA genetic haplotypes with other *L. l. limosa* individuals, they are closely related to other *L. l. limosa* individuals, which might indicate recently restricted gene flow between Swedish *L. l. limosa* and other *L. l. limosa* individuals.

Final thoughts

The aim of this thesis was to use genetics to investigate long term population dynamic processes in Black-tailed Godwit populations resulting from increasing habitat fragmentation or isolation of different breeding habitat on three different spatial scales.

On a global scale my study confirmed the presence of three genetically distinct groups now recognized as subspecies. However, no clear genetic differences were found between *L. l. limosa* across

most of its current breeding range. Possibly, there is some genetic differentiation between breeding areas in the Netherlands and Sweden. In contrast to Dutch Godwit breeding numbers (35.000 breeding pairs), Sweden does not hold large numbers of breeding Godwits (100-250 breeding pairs) (Birdlife International 2004), and Swedish Black-tailed Godwits mainly breed on islands in the Baltic, on Öland and Götland.These populations are also in decline (Birdlife International 2004). So, Sweden, and especially the island of Götland, does seem to harbour genetic information in a very small vulnerable population not found in the Netherlands. If it is one's goal to maintain the full genetic diversity presently available (the whole 'evolutionary potential'), then conservation efforts at Swedish Black-tailed Godwit breeding sites should have priority.

While most research is currently focused on *L. l. limosa* (breeding population requirements, migration routes, and effective management strategies, Kentie *et al.* 2013, Kleijn *et al.* 2010, Lourenco *et al.* 2010, Musters *et al.* 2010, Roodbergen *et al.* 2012) and *L. l. islandica* (migration routes and understanding the ongoing population expansion, Gill *et al.* 2007, Gunnarsson *et al.* 2005;2006) the *L. l. melanuroides* as a subspecies, its breeding areas and its conservational challenges are still largely under exposed. Therefore, I suggest that more research should be done on this subspecies.

Genetic structure in the Netherlands was studied here because Black-tailed Godwit breeding areas seemed geographically fragmented and it was unclear if this had led to genetic separation and inbreeding as well. If genetic structure would have been found this would either have demonstrated isolation of different breeding areas, where genetic drift would have caused either inbreeding and genetic differentiation with other areas, or the presence of founder populations (Frankham 2010). In the case of genetic isolation as a result of habitat fragmentation, management efforts could focus on either maintaining these isolated breeding areas by enlarging them and keep them isolated or connect them directly or indirectly through corridors or translocation. The choice of management action depends on whether the mixing of populations is necessary, for instance when genetic diversity of several breeding populations is low and increasing genetic diversity would thus increase population evolutionary potential. Connecting breeding areas directly by enlarging them would probably be the best strategy in this case.

The Black-tailed Godwit is a species that needs high water tables which are predominantly found in herb rich grasslands. A recent study demonstrated higher densities of breeding Black-tailed Godwits in ´herb rich grasslands´ (Kentie *et al.* 2013). When other populations would harbour high genetic diversity, and connecting breeding areas is not necessarily acute, a long term management strategy could be to enlarge isolated breeding populations (for instance by increasing herb rich grassland habitat), with unique genetic profiles, in order to sustain the genetic diversity and evolutionary potential of the Dutch Black-tailed Godwit population as a whole.

However, all analysis done indicated that habitat fragmentation, although geographically visible, has not yet lead to genetic differences between Dutch breeding populations (not on a local or a national scale). Slatkin (1985, 1987) concluded that only one migrant per generation, which for Black-tailed Godwits is generally considered as 3 years, is needed to obscure any disruptive effects of genetic drift.

On the other hand, Mills and Allendorf (1996) suggest that this number should actually be larger than 1 in many natural populations and that the one migrant per generation rule should be considered as a minimum. Additionally, another study showed that the size of the recipient population(s) under study might also influence the number of migrants needed to avoid excessive inbreeding (Vucetich and Waite 2000). Groen (1993) showed 90% of the adult breeding birds returned within 700 m of the previous nest site. The same research demonstrated natal philopatry to be high as well with 75% of the birds returning within 18 km of their previous hatching site. Demographic research conducted in southwestern Fryslân indicates that around 8% of the adult population formerly captured in that research area disperses beyond 3 km in following years (Kentie *et al.* 2011). Additionally, natal philopatry showed that 5% of the young adults bred beyond 8 kilometers of their former hatching site. The furthest dispersal movement of a young adult within the study area was about 18 kilometers (Kentie *et al.* 2011). There are some colour-ringed birds that have been observed several times in different years at the migration stopover sites but are not reported back at the research area in Fryslân during the breeding season, which could indicate that breeding dispersal outside of the breeding area took place (personal communication with R. Kentie). Kruk *et al.* (1998) showed that on the basis of ring recovery data from the Dutch ringing center between 1900 and 1991 nearly 3% of the natal philopatry took place beyond 50 km. These findings indicate breeding dispersal indeed takes place beyond the borders of the geographically isolated breeding areas, making it likely that the minimum of one migrant per three years does indeed keep different Dutch Black-tailed Godwit breeding areas genetically connected. As such it seems that demographic research does support the idea of a single panmictic population in The Netherlands.

A recent report has studied the Black-tailed Godwit breeding areas ´Black-tailed Godwit core areas´ that have the highest chance of stable population trends, based on soil type, groundwater tables, openness and recent local population trends (Teunissen *et al.* 2012). The main idea is to focus management and financial efforts in these areas instead of trying to preserve all breeding areas including unsustainable and thus futureless areas. A pitfall could be that important genetic diversity might be lost through this way of management, which in turn might impair the species ability to adapt to anthropological and environmental changes (Frankham 2010). Our results indicate that this is not the case on a spatial scale at the size of the Netherlands. In a way the results in this thesis thus support the idea of the ´Black-tailed Godwit core areas´ (Teunissen *et al.* 2012). The most important factors influencing these potential ´core areas´ were demonstrated to be openness, mowing date and watertable (Teunissen *et al.* 2012). Furthermore ´herb rich grasslands´ are demonstrated to be very important for Black-tailed Godwit recruitment (Kentie *et al.* 2013). In general ´herb rich grasslands´ have late mowing dates which increase nest and chick survival directly (Kentie *et al.* 2013, Kleijn *et al.* 2010). Additionally, 'herb rich grasslands' have high water tables, important for the maintenance of Black-tailed Godwit breeding and foraging habitat (Kentie *et al.* 2013, Kleijn *et al.* 2010). Openness, which in some ´herb rich grasslands´ is also quite prominent, is shown to influence nest and chick survival by decreasing predation chance (van der Vliet *et al.* 2008). Our fitness correlation analysis indicated the importance of ´herb rich grassland´ also, and it was demonstrated in a recent study to be great habitat for chicks providing good foraging and hiding opportunities (Kentie *et al.* 2013). Thus most determinants of ´Black-tailed Godwit core areas´ also play an important role

in ´herb rich grasslands´. Therefore focusing management strategies on ´Black-tailed Godwit core areas´ could help with the realization of large scale ´herb rich grassland´ areas. This in turn could potentially increase Black-tailed Godwit recruitment in The Netherlands, which has been decreasing over the last decades (Kentie *et al.* 2013, Schekkerman *et al.* 2008).

Genetics for species conservation

Genetics can be a helpful and insightful tool to investigate population structure and thereby population dynamic processes. In the past decades, genetic approaches have become more efficient in answering ecological questions and therefore more widespread. As a result, the accompanying genetic markers such as allozymes, microsatellites and mitochondrial and nuclear DNA sequences have been used to estimate a wide variety of parameters such as migration rates, population size, kinship, genetic structure and more. Mitochondrial DNA markers and microsatellites have become the most widely used tools in genetic research. Both were used in the research making up this thesis. However, although most of the genetic studies have used and are still using one or both of these markers to study genetic patterns in natural populations (Liebers *et al.* 2004, Ottval *et al.* 2005, Paton *et al.* 2002, Ronka *et al.* 2008, Zink and Barrowclough 2008, Zarza *et al.* 2011), the shortcomings which inherit both markers might severely impair the conclusions that can be drawn for species conservation or in the field of conservation biology currently.

Mitochondrial DNA is present in most cells in high copy number, making it easy to isolate mtDNA, and is relatively rapid and inexpensive to sequence. After sequencing, a phylogenetic tree of mtDNA haplotypes (variable genotypes of the mtDNA), rooted with a closely related taxon, will reveal whether closely related haplotypes occur locally or throughout the range and form a picture of the genetic structure. However, several problems have been defined since this marker type started to be used for studies of genetic structure. First, the mtDNA is one locus, which means that every gene that lays on the mtDNA evolves in a linked fashion together with the other genes on the mtDNA, with only one gene tree as a result irrespective of the amount of genes one would sequence. This might not give an adequate representation of the genetic structure present at the genome level of an individual. In other words, even if many genes of the mtDNA are sequenced the sample size for that one individual will always be one. An additional concern with mtDNA is the fact that it is maternally inherited, which entails that the structure found only portrays the female lineage. If a population would maintain gene flow through males only, the genetic structure in the mtDNA could give a wrong representation of lineage history. A third problem is base substitutions on the same base, which could potentially mask deeper lineage diversification (Zink and Barrowclough 2008, Edwards and Bensch 2009). The usage of nuDNA, sequenced at multiple inlinked loci, or unlinked microsatellites can solve some of these problems. nuDNA is both maternally and paternally inherited. Furthermore, by sampling a large amount of unlinked nuclear loci, which is possible with nuDNA, one improves the large confidence intervals otherwise associated with mtDNA phylogeographic analysis. Although issues concerning homoplasy have not yet been empirically tested, homoplasy is believed to pose problems in nuDNA sequence analysis as well as in microsatellite analysis (Selkoe and Toonen 2006, Zink

and Barrowclough 2008). Additionally, nuDNA has a serious impairment compared to mtDNA when it comes to coalescent times. Due to the fact that nuDNA is inherited through both parents and recombines during mitosis lineage sorting on average takes 4 times, termed $4N_e$, longer for nuDNA than for mtDNA. In general this phenomenon results in mtDNA being able to portray signals of lineage diversification much quicker than nuDNA can (Zink and Barrowclough 2008).

With this in mind one should probably consider mtDNA gene trees as the backbone of a genetic structure or phylogeographic study. Subsequently, nuDNA can be used to confirm the mtDNA signal and make CI's in several analysis smaller. However, in that case one does not consider that the mtDNA signal itself (although being able to show changes in genetic structure more quickly) being one locus only, might not be the general representation of population genetic structure on a genome level (Edwards and Bensch 2009). Should one consider different randomly dispersed loci with long coalescence times as indicative of a genetic pattern in a population or is one locus that is maternally inherited and has much faster coalescence time respectfully, better? In essence one also needs to address the following question: should we always look for as much genetic structure as possible; does the genetic signal with the most structure always give the most thorough representation of the populations under study?

Luckily, new techniques are arising on the horizon, which might be able to overcome the shortcomings of mtDNA and nuDNA for conservation genetic research. Next generation sequencing rapidly generates huge amounts of sequence data in a very cost-effective way making it possible for molecular ecologists to get a genome wide representation of genetic structure in non-model organisms (Ekblom and Galindo 2011, Jakobsson *et al.* 2008, Novembre *et al.* 2008). This technique has the potential to enhance the scale and detail of population genetic research enormously and make it possible to answer questions concerning habitat fragmentation and quality very thoroughly. Only small amounts of DNA are needed for detailed genetic analysis, which is helpful in difficult to sample species. Additionally, where currently only a handful of genes or microsatellites are used to answer conservation related questions the rise of NGS enables the shift from conservation genetics to conservation genomics. Potentially hundreds or thousands of genes can be studied possibly involved in phenotypic variation or selection (Ekblom and Galindo 2011, Ouborg *et al.* 2010). DNA sections under selection, generally considered to show very fast lineage sorting, could additionally be used to study, for instance, recent habitat fragmentation. However, with the rise of this very promising technique new problems arise. How to store thousands of terra or even peta bytes in a cost-effective way, and what does all the data mean from an ecological or conservation point of view? It is clear we are not quite there yet. Future challenges most likely lay in bioinformatics and interpreting all the genomic data to answer ecological and conservation related questions. Surely, the next decades are going to show some very exciting changes in genomics and related fields such as conservation genetics.

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