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Title: Genetic patterns of Black-tailed Godwit populations and their implications for conservation
Issue Date: 2013-11-05
CHAPTER 1

General Introduction
Introduction

The 20th century intensification of agricultural land use in Europe has lead to steep increases in agricultural yield (de Snoo et al. 2012, Stoate et al. 2001; 2009). In The Netherlands, most of the low-productive grassland areas have been converted to highly productive agricultural grasslands by applying artificial fertilizer and pesticides, by lowering groundwater levels, and by sowing nutritionally demanding fast growing varieties of grass (de Snoo et al. 2012, Stoate et al. 2001; 2009). Consequently, flora and fauna diversity in many grassland ecosystems have decreased dramatically (Beintema et al. 1995, Bijlsma et al. 2001, Donald et al. 2001; 2006). In this thesis the Black-tailed Godwit *Limosa limosa*, a flagship Dutch farmland bird, was chosen as a model organism to further investigate 2nd and 3rd order effects on biodiversity as a result of the agricultural industrial revolution in the 20th century.

The Black-tailed Godwit is a rather large wader, which can be distinguished by their long bills and legs, the white lower rump contrasting with a black tail-tip and the white stripes across the dark upper wings (http://en.wikipedia.org/wiki/Black-tailed_Godwit - cite_note-collins-2). The Black-tailed Godwit breeds from late March to late June. Three subspecies have currently been described, *L. l. islandica*, *L. l. limosa*, and *L. l. melanuroides*. The nominate *L. l. limosa* breeds from Great Britain and Central Europe to Central Asia and Asiatic Russia as far east as the river Yenisey (Cramp & Simmons 1982). The islandic subspecies breeds in Iceland, small populations occur on the Faeroe Islands, Shetland and the Lofoten Islands. The *L. l. melanuroides* breeds in Mongolia, Northern China and the Russian Far East.

After the *L. l. limosa* young fledge, breeding birds begin to congregate in flocks, which migrate southwards between late-June and October. During the autumn migration Black-tailed Godwits may roost in large flocks (sometimes tens of thousands) in rice fields in Spain and Portugal, and a proportion of the adults pause in North Morocco in July to moult (Cramp & Simmons 1982). The Black-tailed Godwit winter across a broad range from the Republic of Ireland to Australia, encompassing the Mediterranean, sub-Saharan Africa, and parts of the Middle East, India, China, Taiwan, the Philippines, Indonesia, and Papua New Guinea (Birdlife International 2012). West European Black-tailed Godwits of the *limosa* subspecies winter in Africa North of the equator and south of the Sahel, Guinea-Bissau, the coastal zone of Southern Senegal, the Senegal delta, Mali at the Inner Niger Delta, the Chad Basin (Zwarts et al. 2009). An increasing number of West European Black-tailed Godwits winter in Spain and Portugal (Lourenço et al. 2010). East European breeding Black-tailed Godwits winter in the eastern part of the Sahel and East Africa (Cramp & Simmons 1982, Zwarts et al. 2009). Northward migration takes place between February and April, and birds often arrive at the breeding grounds in large groups.

Current status with a Dutch historical perspective

The European stronghold of the *L. l. limosa* subspecies is found in The Netherlands where 40,000-50,000 Black-tailed Godwit breeding pairs were estimated to breed in 2004 (Birdlife International 2004). In 2004 breeding numbers of *L. l. limosa* were estimated at at 162,000-183,000 in western Europe, 90,000-165,000 in eastern Europe, 25,000-100,000 in West-Central Asia, 150,000 in
Central Asia and Russia (Birdlife International 2004). A still increasing population of 47,000 pairs of *L. l. islandica* breeds in Faeroes, Lofoten and Iceland (Gunnarsson *et al.* 2005). The *L. l. melanuroides* populations are estimated at 160,000 breeding pairs that reside in parts of Eastern Asia (Birdlife International 2012). Until the Middle Ages Black-tailed Godwits probably were not very abundant in The Netherlands. Archeological studies did find bones, in small amounts, of Black-tailed Godwit in food material from 700 B.C. until 1700 (Prummel & Heinrich 2005, Zeiler & Brinkhuizen 2003). Before the Middle Ages Black-tailed Godwit breeding habitat in the Netherlands consisted mainly of raised bogs, moorlands, lake margins, damp grassy depressions in steppe (Beintema *et al.* 1995, Haverschmidt 1963). When raised bogs were converted to wet grasslands and hay lands, during and after the Middle Ages, for the purpose of dairy farming, the Black-tailed Godwit probably shifted from their remaining original breeding habitat to these newly formed habitats (Beintema *et al.* 1995, Haverschmidt 1963).

*Figure 1:* Land use in The Netherlands during 1900 on the left, and 2000 on the right (Knol *et al.* 2004). Colours depicted indicate: grass/hayland (light green), arable/unused land (sand colour), heath/raised bogs (purple), forest (dark green), city/roads (red), dunes (yellow), swamp (pink), water (blue), remaining (grey).
From that time onwards Black-tailed Godwits would be known as breeding birds of meadows: meadow birds. One of the first Dutch ecologists, J. Drijver wrote in ‘De Levende Natuur’ in 1914: “I highly appreciate the Black-tailed Godwit as a meadowbird...”. From 1900 on the Black-tailed Godwit was mostly present as a breeding bird of the damp hay lands, which were the major part of The Netherlands (figure 1). Here the Black-tailed Godwit profited from the usage of artificial fertilizer, in turn increasing food availability, which increased between 1900 and the 1980s (CBS 2001;2008,). This was probably the reason for the increase in Black-tailed Godwit population numbers in these habitats during the first half of the 20th century (Beintema et al. 1995, Havenschmidt 1963, Schroeder et al. 2010). However, with further agricultural changes population numbers stagnated in the late 1960s when the first comprehensive survey of this breeding bird in The Netherlands yielded an estimate of 120,000 pairs of Black-tailed Godwits in The Netherlands (Mulder 1972). Population numbers have declined ever since with an annual rate estimated at 5% (Bijlsma et al. 2001, Gill et al. 2007). The available information about Black-tailed Godwit trends indicates that the global population has probably declined between 14% and 33% over the past 15 years (Birdlife International 2012). Consequently, the IUCN has currently qualified the species as Near Threatened (Birdlife International 2012).

**Reasons for the decrease**

Agricultural intensification at the breeding areas is deemed by far the most important negative influence on Black-tailed Godwit breeding numbers (Gill et al. 2007, Beintema et al. 1995, Hustings & Vergeer 2002, Schroeder et al. 2010, Kentie et al. 2011). However, several other factors outside and at the breeding areas, such as hunting, predation, urbanization and the decrease of agricultural grassland area due to the conversion of grassland to cornfields, have probably also impacted Black-tailed Godwit population numbers negatively during the second half of the 20th century.

1) **Outside the breeding area: hunting**

During migration and wintering, hunting poses an additional threat to Black-tailed Godwit adults and fledglings (van Noordwijk & Thomson 2008, Zwarts et al. 2009). In France it used to be allowed to shoot Black-tailed Godwit individuals during migration, however since 2008 the EU has implemented a five year ban on hunting the species (Birdlife International 2012). Zwarts et al. (2009) report that there is a general increase in the number of Balanta rice farmers in Guinea-Bissau that perceive Black-tailed Godwits as a pest. Since many Black-tailed Godwits now return to Africa earlier, that damages the local crops especially rice crops. This has led to some birds being shot in their wintering grounds (Zwarts et al. 2009). However, the precise scale and impact of Black-tailed Godwit hunting in West-Africa are still unknown. Additionally, drought in the West African wintering quarters may have had negative impacts on the mainland European population (Zwarts et al. 2009). As a result of drought, foraging opportunities decrease which makes it easier for hunters to shoot Black-tailed Godwits on their West African wintering grounds (Zwarts et al. 2009).

2) **At the breeding area: decrease of habitat quality**

In regards to meadowbirds, agricultural intensification can be summed up by the following: the lowering of the ground water tables, increased sowing of fast growing grass species, higher fertilizer usage, the usage of more life stock, earlier mowing dates and the increase of mowing frequencies.
Agricultural intensification practices are all directed towards increasing productivity. Lower groundwater tables negatively affect the availability of prey items for Black-tailed Godwit adults and chicks during the breeding season (Kleijn et al. 2009; 2010, Verhulst et al. 2007).

Earthworms which form the most important food source for adult Black-tailed Godwits generally follow the groundwater table (Siepel et al. 1990). When groundwater tables are lowered earthworms become less available for the foraging birds (Kleijn et al. 2009). This might cause adults to postpone breeding, which in turn might negatively influence egg size during nesting (Schroeder et al. 2012). Moreover, several studies show that small egg size is negatively correlated with chick body condition and survival (Arnold et al. 2006, Blomqvist et al. 1997, Bolton 1991). Dry soil warms up quicker, resulting in a faster growth of the grass vegetation. In combination with using fast growing grass species, high fertilizer input, and the introduction of the monoculture, the grass swards form tall and dense swards which negatively affects foraging capabilities for Black-tailed Godwit chicks (Kleijn et al. 2010, Schekkerman et al. 2008). Production benefits from this denser and higher grass vegetation as it allows for earlier and more frequent mowing during spring. Agricultural mechanization has further increased production allowing for faster mowing of larger meadows. As a result current mowing dates have advanced, while the timing of breeding has stayed the same. This poses a direct danger for meadowbird reproduction, see figure 2 (Beintema et al. 1995, Kleijn et al. 2010, Musters et al. 2011).

Furthermore, mowing has been demonstrated to decrease the availability of arthropod abundance in the freshly mown fields, increasing chick mortality through starvation (Kleijn et al. 2010, Schekkerman & Beintema 2007). An additional problem with earlier mowing is the reduced cover that chicks have to deal with on the freshly mown fields. This might increase chick predation significantly (Schekkerman et al. 2009, Teunissen et al. 2005). Moreover, the increase of meadowbird predators, Northern Goshawk (Accipiter gentilis), Common Buzzard (Buteo buteo), small carnivorous mammals and probably the Red Fox (Vulpes vulpes) in general might also negatively influence Black-tailed Godwit recruitment (Teunissen et al. 2005; 2008). Like in large parts of the world, urbanization in The Netherlands has increased dramatically over the last 100 years (figure 1). Increased urbanization might further increase predation as potential predators like jackdaws (Corvus monedula), house cats and dogs have easier excess to the meadows and as such decrease habitat quality.

3) **At the breeding area: habitat fragmentation**

As a result of decreasing habitat quality the areas with breeding Black-tailed Godwit have become fragmented. Moults and skins of the Black-tailed Godwit collected between 1826-1935 show that surprisingly many individuals were found in or close to the present day core breeding areas (Schroeder et al. 2009; figure 3a). Black-tailed Godwit breeding areas seem to be mostly connected in 1967 (figure 3b). Currently, it is clear that the Black-tailed Godwit breeding landscape is better described by fragments of breeding sites surrounded by unoccupied areas (figure 3c; d). Small fragments of habitat can only support small populations of plants and animals. Furthermore, small populations are more vulnerable to extinction. Minor fluctuations in climate, resources, or other factors could be catastrophic in small, isolated populations where they would have been insignificant and quickly
corrected for in large populations (Hanski 1999). For mobile species, like birds, the dangers of habitat fragmentation are generally seen as less important. However, the effect that habitat fragmentation can have on the population dynamics of a species is not only dependent on the mobility but also on the breeding site faithfulness of a species (Hanski 1999). The Black-tailed Godwit is a highly breeding site faithful bird with high adult nest site fidelity and some natal philopatry (Groen 1993, Kentie et al. 2011, Kruk et al. 1998, van den Brink et al. 2008). Groen (1993) showed 90% of the adult breeding birds returning within 700 m of the previous nest site. Natal philopatry was demonstrated as well with 75% of the birds returning with 18 km of their previous hatching site. 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. This combination of high breeding site faithfulness and continuing habitat fragmentation of Black-tailed Godwit breeding populations could pose a serious threat to the long term survival of the species.

![Figure 2](image.png)

*Figure 2.* The median hatching date of the Black-tailed Godwit and the median mowing date of dairy farm grasslands in The Netherlands from 1980 to 2005 (Kleijn et al. 2010).

Urbanization, next to the increased planting of trees and vegetation encroachment in the agricultural meadows which might decrease habitat quality, might further influence Black-tailed Godwit populations negatively as it decreases landscape openess and the agricultural grassland areal (Melman et al. 2008, van der Vliet et al. 2008;2010). This might further increase habitat fragmentation for breeding Black-tailed Godwits. It remains unclear if this combination of high breeding site faithfulness and the increasing amount of habitat fragmentation is currently causing isolation of Black-tailed Godwit breeding areas.
Figure 3: Black-tailed Godwit distribution through time a) The distribution of Black-tailed Godwits between 1826-1935, distilled from moults and skins of Black-tailed Godwits that were collected on different locations in the breeding season during this time, b) the Black-tailed Godwit distribution map from 1967 (Mulder 1972), c) the Black-tailed Godwit distribution map from 2005 (based on the ‘Gruttokaart’ Teunissen et al. 2005), d) a local Black-tailed Godwit distribution map from the southwestern part of Fryslan 2011 (Kentie et al. 2011).
4) **The proportion of the population affected by the decrease**

It seems that the current negative Black-tailed Godwit population trends mainly result from the decreased recruitment during breeding. Research on Black-tailed Godwit adult survival in The Netherlands demonstrates an absence of clear changes over the last 25 years (Roodbergen et al. 2008, 2012, Schekkerman et al. 2008). Demographic studies conducted on Dutch Black-tailed Godwit populations, which estimated adult survival and which kept sample size and ringing effort stable throughout the study period, demonstrated adult survival in recent years to be similar to adult survival in 1960, lying around 0.8 (Beintema & Drost 1986, Groen & Hemerik 2002, Kentie et al. 2011, Noordwijk & Thomson 2008, Roodbergen et al. 2008, Schekkerman et al. 2008). Contrastingly, like Black-tailed Godwit population trends, chick recruitment has been showing a steady decrease since 1980 (Schekkerman et al. 2008).

Altogether, these agricultural changes, urbanization and decrease of available grassland area, seem to have led to the decrease in habitat quality and the fragmentation of suitable grassland, in turn decreasing Black-tailed Godwit recruitment (Gill et al. 2007, Kentie et al. 2013, Schekkerman et al. 2008, Zwarts et al. 2009).

**Policies to stop the decrease**

To halt and reverse negative Black-tailed Godwit population trends, several management strategies have been formulated and have been implemented throughout the 20th century. In 1909 the conservation agency ‘Natuurmonumenten’ already instigated grassland reserves for the purpose of meadow bird preservation (Beintema et al. 1995). Since then the surface of grassland reserves have increased enormously (Dekker 2002). A second form of meadow bird management is the protection of the nests by volunteers. Nest protection increases clutch success by twofold and is especially effective for nests found on grassland and crop farms that would otherwise be destroyed by agricultural activities and trampled by cattle (Oosterveld et al. 2007). Presently, over 150,000 meadowbird nests are protected by volunteers (Goedhart et al. 2010). Around 2000, an alternative management technique was introduced to stop the rapid decline of meadowbirds in the Netherlands, called ‘mosaic management’, as it became increasingly clear that increasing clutch survival alone could not halt the negative Godwit population trends. Higher recruitment was needed, which could be obtained by providing suitable foraging habitat for adults during the nesting period and chicks after hatching; grassland with high water tables for foraging adults and grass with the suitable density and height supplying chicks with enough food and cover, up to fledging (Oosterveld et al. 2011). The most important characteristics of ‘mosaic management’ are an implementation on a landscape level and a specific spatial distribution of this grassland mosaic within the area, with the purpose to meet all the ecological requirements of the meadow birds throughout the breeding season (Oosterveld et al. 2011). Several forms of mosaic management, such as postponed mowing, adapted grazing, an increase of the amount of water on the production land to increase foraging possibilities for meadowbirds and using hay rich farmyard manure instead of semi liquid manure to increase a more open and lower meadowbird grassland habitat have been used in different compositions throughout The Netherlands. Farmers that agree to join mosaic management are compensated for the loss in production (Oosterveld et al. 2011).
Lack of knowledge

It remains questionable if these different management strategies are adequate to sustain Black-tailed Godwit populations. As a management type, buying and managing new grassland reserves optimally for meadow birds has slowed down. Nest protection is an adequate measure to increase clutch success in many cases but clutch success alone does not improve recruitment. Furthermore, in areas with high predation pressure nest protection often may have negative effects on clutch survival (Goedhart et al. 2010). It was shown that the number of nest visits increases predation success by nearly 10% per visit in areas with high predation pressure since voluntary nest protectors leave trails or body scents directed towards the nests, which might be easy to follow by predators (Goedhart et al. 2010). Furthermore, mowing around protected nests leaves little islands of grass around these nests in an otherwise mown field, which might also increase predation success (Kentie et al. 2011). Mosaic management results (which in The Netherlands form an important part of the agri-environmental schemes) are still difficult to interpret, as its efficiency is highly dependent on the combination of mosaic management types implemented, the focal species, the weather conditions during the breeding season and local external factors like predation pressure and the amount of disturbance (Kleijn & Sutherland 2003, Kleijn et al., 2006, Oosterveld et al. 2007;2011, Verhulst et al., 2007, Willems et al., 2004). Up till now it has still not been scientifically demonstrated that these measurements have the desired effect (Oosterveld et al. 2011). However, to know whether mosaic or any other form of conservation management has effect on meadowbird populations on the landscape level, one needs to determine if these measurements can improve habitat quality in terms of recruitment and consequently decrease habitat fragmentation.

By using capture mark recapture techniques (CMR) a broader understanding of the quantity and consequence of increasing habitat fragmentation and decreasing habitat quality can be acquired. CMR techniques can be used to study population dynamic aspects like, source-sink dynamics, dispersal, breeding site faithfulness, natal philopatry, the fraction of birds that breed and yearly survival of the population, which in turn can be used to quantify habitat fragmentation or determine habitat quality (Bouwhuis et al. 2012, Kentie et al. 2011, McGowan et al. 2009). CMR studies come at the cost of being time consuming, which limits the spatial and temporal scale across which they can be carried out. This may hinder the extrapolation to non studied areas.

Another way to study the effects of habitat fragmentation and habitat quality is through genetics. Habitat fragmentation and changes in habitat quality might affect the population dynamics of a species. In turn the resulting population dynamic processes, such as a metapopulation structure or sources and sinks might have an effect on the genetic pattern of the species. Accordingly, these genetic patterns can be studied through conservation genetics. Conservation genetics focuses on the genetic consequences arising from reduction of once large, outbreeding populations to small units, as a result of for instance deteriorating habitat quality or habitat fragmentation, where stochastic factors and the effects of inbreeding are profound. It encompasses the use of genetic theory, derived from the field of population genetics. Through the field of conservation genetics, genetic processes such as inbreeding, genetic drift, the loss of genetic diversity, and gene flow can be studied, all of
which might be informative about the population dynamics of the species under study (Frankham et al. 2010). Subsequently, this type of genetic research can help with the reasoning of why and how a species should be protected. This can be done through resolving taxonomic uncertainties, defining management units, resolving fragmented population structures, identifying species or populations at risk due to low genetic diversity and defining sites for reintroduction (Frankham et al. 2010). As it is much less labour-intensive than the marking and following of cohorts of individual animals over their lifetime, conservation genetic research can be conducted on larger spatial scales. This might lead to a better understanding of habitat fragmentation and quality on the landscape level. Additionally, genetic changes that can potentially result from habitat fragmentation and changes in habitat quality take place during a much longer time frame than those that can be researched with CMR studies. This could increase the insight in historical population changes when using genetics.

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. However, before genetic research could be conducted genetic markers and easy sampling methods were needed. Therefore, microsatellite markers were developed. Additionally, the potential of a DNA source other than blood was investigated for the rest of the genetic research. After this methodological research this thesis focuses on answering the next questions.

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

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

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

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

To investigate this we focus on three spatial scales: regional, national and global.
Outline of the thesis

This thesis is composed of 6 research chapters and a general discussion.

In chapter 2 we used the developed microsatellite markers to test egg shell membranes as an alternative sampling method to blood samples in population genetic research. Collecting egg shell remains is less time consuming than collecting blood samples and more DNA samples can be gathered, by collecting egg shells, during the same time span compared to blood samples. We focused on contamination issues, the DNA yield and purity of egg shell membrane derived DNA and compared the results to DNA isolated from blood samples.

With the developed markers and additional sampling method generated from these methodological studies, further genetic research could be conducted. These will be discussed in chapters 4 to 6.

In chapter 3 we re-examine the very interesting findings of a previous study that demonstrated intronic variation in the CHD1-Z gene in Black-tailed Godwits to be correlated with fitness using a much bigger dataset based on additional years of the previous CMR study. The previous study suggested that Black-tailed Godwit breeding populations were differentially structured at different habitat types as a result of positive selection pressures on birds breeding at extensively managed (generally considered to be of high habitat quality to Black-tailed Godwits) agricultural grassland. Here we re-examined these findings of possible population structure correlated with habitat quality on a local scale. Furthermore, we investigated several other fitness correlates.

Chapter 4 describes the results of the population genetic structure of Dutch Black-tailed Godwit breeding populations. Nine different breeding locations entailing most of the Black-tailed Godwit breeding range in The Netherlands were analyzed for population structure, F-statistics, genetic diversity, genetic differentiation, inbreeding, isolation and gene flow.

Chapter 5 re-examines the genetic differentiation between these nine Black-tailed Godwit breeding sites using a relatively new form of statistics called D. This statistic is less dependent on heterozygosity and within population diversity to determine genetic differentiation between populations and as such might yield different results compared to the traditional F-statistics.

In chapter 6, the genetic population structure and phylogeny of the Black-tailed Godwit was assessed, entailing DNA samples ranging from Iceland to Lake Baikal in eastern Russia and including all three subspecies of Black-tailed Godwit. A historic explanation is given for the genetic patterns found in both microsatellites and newly developed mtDNA markers.

Finally, in chapter 7 the results and subjects of the previous chapters are discussed. In this general discussion we explore the usefulness of our findings and population genetic structure studies for the conservation of Black-tailed Godwit populations and animal conservation in general.
References


