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

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CHAPTER

4

No evident spatial genetic structuring in the rapidly declining Dutch Black-tailed Godwit *Limosa l. limosa* population

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Abstract

With 40% of the European Black-tailed Godwit population breeding in The Netherlands, this country internationally harbours important numbers of this species. However, ongoing agricultural intensification has resulted in the fragmentation of the population and drastic population declines since 1967. Establishing genetic diversity, genetic differentiation and gene flow on the basis of 12 microsatellites, we investigated whether the population genetic structure of the Dutch Black-tailed Godwit bears the marks of these changes. Genetic diversity appeared moderate and Bayesian model-based analysis of individual genotypes revealed no clustering in the Dutch populations. This was supported by pairwise F_{ST} values and AMOVA, which indicated no differentiation among the nine separate breeding areas. Gene flow estimates were larger than "one migrant per generation" between sample locations, and no isolation by distance effect was demonstrated. Our results indicate the maintenance of moderate levels of genetic diversity throughout the Dutch Black-tailed Godwit population through appropriate levels of gene flow between different breeding areas. We suggest that the Dutch Black-tailed Godwit breeding areas should be managed as a single panmictic unit, much as it is presently done.

Keywords Genetic differentiation · Genetic diversity · Gene Flow · Habitat fragmentation · Microsatellites · Negative population trends

Introduction

For a long time agriculture in Europe generated high bird species diversity, but with increasing intensification over the last 50 years, many species now show seriously negative trends (Chamberlain *et al.* 2000, Stoate *et al.* 2009, Teunissen and Soldaat 2005, van Turnhout *et al.* 2007). A good example of a bird species that waxed and waned in response to agricultural land use changes is the Black-tailed Godwit *Limosa limosa limosa* (Bijlsma *et al.* 2001, Birdlife International 2009). The Black-tailed Godwit was previously confined to raised bogs, moorlands, lake margins and damp grassy depressions in steppe. However when wet grassland created for the purpose of dairy farming increased in northwestern Europe, this species became very successful through exploitation of this habitat (Beintema *et al.* 1995, Haverschmidt 1963). Close to half the European Black-tailed Godwit population was known to breed in The Netherlands (Birdlife International 2004, Teunissen and Soldaat 2005). However, continuing declines of 5% per year since the peak numbers of the late 1970s (Schroeder *et al.* 2009) have decreased Black-tailed Godwit breeding numbers. While 120,000 pairs (Mulder 1972) were estimated to breed in 1967, only 40,000 pairs remained in 2004 (Teunissen and Soldaat 2005).

The most significant threats for this species include loss of nesting habitat owing to wetland drainage and agricultural intensification. The earliest modernization of farming enhanced food supply and thus increased population sizes of several wader species (Cramp and Simons 1983). However, further intensification practices have resulted in reduced food availability, lower water tables, increased cattle densities, and increased early mowing (Benton 2001, Bijlsma *et al.* 2001, Schekkerman *et al.* 2008). Furthermore, predation risk has increased as a result of early mowing practices, mostly due to reduced coverage for nesting, and chick raising (Schekkerman *et al.* 2009). These agricultural adjustments have in turn culminated in impaired chick recruitment and decreasing habitat quality. Subsequently, the declining habitat quality has led to the fragmentation of suitable grassland (Teunissen and Soldaat 2005, Zwarts *et al.* 2009). Schekkerman *et al.* (2008) documented a decline from 0.9 fledged chicks per godwit pair in 1985 to roughly 0.23 fledged chicks per pair in 2006.

Although, Black-tailed Godwit habitat is becoming more fragmented and habitat quality decreasing, this species shows high breeding site fidelity and some natal philopatry (Groen 1993, van den Brink *et al.* 2008). Groen (1993) showed 90% of the adult breeding birds returned within 700 m of the previous nest site. These results are supported by van den Brink *et al.* (2008) who found that 100% of the adult Black-tailed Godwits returned within 3 km of the former nest site. Natal philopatry was demonstrated to be high as well with 75% of the birds returning within 18 km of their previous hatching site. With adult dispersal being limited and the number of chicks dispersing beyond 18 km theoretically decreasing due to declining recruitment, breeding sites could have become partly or completely isolated from each other. This might affect population dynamics, resulting in a metapopulation structure including source-sinks or isolation by distance (Höglund 2009).

Genetic connectivity between areas is maintained by dispersal of successfully reproducing animals among breeding areas, i.e. gene flow. Slatkin (1985, 1987) concluded that only one migrant per generation 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).

Here we studied gene flow indirectly by investigating genetic diversity and differentiation on a national scale covering the most important breeding areas of the Dutch Black-tailed Godwit breeding population (Figure 1). 12 microsatellite loci developed specifically for this species by Verkuil *et al.* (2009) were used. First, the markers were validated to evaluate if they were polymorphic and were in congruence with the assumptions made by several population genetic software. Second, the population genetic structure of the Dutch Black-tailed Godwit breeding sites was assessed through genetic diversity, genetic differentiation and gene flow calculations. Moreover, if genetic structure (isolation of breeding areas with genetic differentiation between areas and or low genetic diversity) or the lack of it was portrayed, we tried to explain the underlying mechanism.

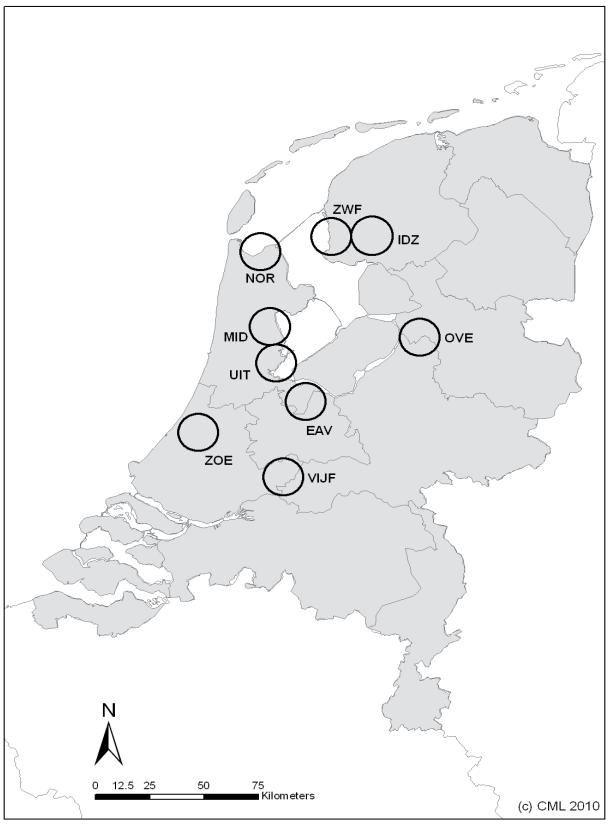

Figure 1. Sample locations; Zuid-West Fryslân (ZWF), Eemnes/Arkemheen/Vinkeveen (EAV), Zoeterwoude (ZOE), Idzegea (IDZ), Middelie (MID), Normerpolder (NOR), Vijfheerenlanden (VIJF), Uitdam (UIT) and OVE (Overijssel).

Material and methods

Sample collection, DNA extraction and amplification

From 2004 to 2008, blood samples from freshly hatched chicks and dry egg shell remains (Trimbos *et al.* 2009) were collected in nine distinct regions in The Netherlands (Figure 1). Regions were from 7 to 135 km apart. With the help of voluntary nest protectors, nests were located early during incubation, through continuous observational effort. When a nest was found, the floating method of Liebezeit *et al.* (2007) was used to determine hatching date. Around the hatching date the nests were visited daily to obtain either eggshells or ca. 30 µl whole blood per chick. To minimize post-sampling contamination, individual egg shells were stored in plastic bags. Blood was stored in individual 1.5 ml Eppendorf tubes containing 97% alcohol buffer. Blood samples were stored at -70 ˚C, while egg shells were stored at room temperature to get a good separation of the egg shell membranes from the outer shell. DNA samples of 140 individual Black-tailed Godwits were collected (Table 1). These 140 samples incorporated only one individual per nest to keep relatedness between samples as low as possible.

DNA was extracted from 6-10 µl of blood using the Ammonium Acetate method as described by Richardson *et al.* (2001). DNA was extracted from eggshell membrane using Qiagen Dneasy Tissue Kit (Qiagen 2003), with minor modifications as described by Trimbos *et al.* (2009). DNA quality and quantity were checked twice, using the NanoDrop ND-1000 (Thermo Scientific) for 260/280 ratios and concentration values. For optimal PCR amplification, blood samples were diluted to concentrations below 10 ng/µl. Compared to blood derived DNA, DNA from eggshell membranes was of less purity occasionally. Consequently, eggshell derived DNA was diluted to concentrations below 50 ng/µl. We used 12 microsatellite loci (LIM3, LIM5, LIM8, LIM10, LIM11, LIM12a, LIM24, LIM25, LIM26, LIM30, LIM33) developed for Black-tailed Godwits (Verkuil *et al.* 2009). The final volumes of the PCR amplification mix were 11 µl and included 1-10 ng DNA for blood samples or 1-50 ng DNA for eggshell membrane samples, 1.65 mM $MgCl_2$, 2.5 µM dNTPs, 0.5 µM forward primer with M13 extension, 0.5 µM reverse primer, 1 µM fluorescent-labelled M13 primer, 10x PCR buffer and 0.45 U Taq DNA Qiagen polymerase. The polymerase chain reaction program used was as described by Verkuil *et al.* (2009), except the final PCR step was extended to 20 min to minimize peak stutter patterns. PCR products were analyzed using a MegaBACE 1000 (Amersham Biosciences) and allele sizes were assigned using Fragment Profiler 1.2 (Amersham Biosciences 2003). Contamination of PCR pre-mix with exogenous DNA was minimized by carrying out pre- and post-PCR pipetting in different rooms. Additionally, to control for potential contamination problems, negative controls were included in every PCR reaction and MegaBACE runs.

Genetic marker validation

Microsatellite markers are expected to be independently distributed in the genome as such linkage between loci would result in pseudo-replication (Selkoe *et al.* 2006). A Fisher's exact test for linkage disequilibrium was carried out using the samples from the nine breeding areas, with 1,000 dememorization steps, 100 batches and 1,000 iterations per batch (GENEPOP; Raymond and Rousset 1995, web version 4.0). Deviations from Hardy-Weinberg, heterozygote excess and deficit were tested per

locus and sample location separately using 1,000 dememorization steps, 100 batches and 1,000 iterations per batch (GENEPOP; Raymond and Rousset 1995). For multiple testing Bonferroni correction was applied (Rice 1989). MICRO-CHECKER was used to test for scoring and amplification errors (stutter and null alleles) with a 95% confidence interval over 10,000 runs (Oosterhout *et al.* 2004). To evaluate genotyping error, scoring was performed three times and the frequency of disagreement between different times of scoring was noted and averaged.

Genetic diversity, F_{IS} and population structure analyses

Observed (H_o), expected heterozygosity (H_e), inbreeding values (F_{IS}) per location and pairwise F_{ST} between locations were calculated using ARLEQUIN 3.11 (Excoffier *et al.* 2005). Furthermore, an analyses of molecular variance (AMOVA) was performed, through which variance among sample locations (V_a), among individuals within sample locations (V_b), and within all individuals could be computed (V_c), using ARLEQUIN with 20,000 permutations. If significant values were obtained, Bonferroni correction was applied. Number of private alleles was determined using CONVERT 1.31 (Glaubitz 2004). FSTAT 2.9.3.2 (Goudet 1995) was used to calculate allelic range, number of alleles per sample location and allelic richness per sample location. This program uses the rarefaction index, as described by Hurlbert (1971), to correct for sample size. Additionally, the levels of allelic richness and F_{IS} among sample locations were compared using FSTAT with 10,000 permutations to obtain P-values.

The model based Bayesian cluster algorithm implemented in STRUCTURE 2.3.1 (Pritchard *et al.* 2000) was used to cluster from a pool of genotypes from all sampling locations. We determined the deltaK (Structure Harvester) which is a calculation of the second order rate of change in log likelihood Ln P(X|K) recommended by Evanno *et al.* (2005). Although this method was demonstrated to be more reliable in estimating the inferred amount of clusters in natural populations, K = 1 cannot be measured which was a reasonable possibility for this research. Consequently, the most likely number of genetic clusters (K) in our sample set was also investigated by determining the maximum average log likelihood Ln P(X|K). Values computed with both methods were plotted using Structure Harvester 0.56.3 (Dent 2009, web version). The Structure model was run using admixture and correlated allele frequencies. Additionally, the LOCPRIOR model, incorporated into STRUCTURE 2.3.1, was used. This model assumes that individuals sampled close together are often from the same population and can assist in the clustering when population structure is weak. The program was run 5 times with a burn-in period of 200,000 iterations and a length of 1,000,000 MCMC iterations for K (1-11). Convergence was checked by looking whether the graphs provided by the program reached equilibrium before the end of the burn-in phase.

The genetic structure profile within this dataset could display a historic situation of Black-tailed Godwit population dynamics. The Dutch Black-tailed Godwit breeding population is believed to have expanded from 1900 until the 1960s. The k test as implemented in the program Kgtests (Bilgin 2007) detects population expansion on the basis of allele size distributions. The method uses a one tailed binomial distribution to test for the number of loci with negative k values and if this represents a significant number of negative k values. Additionally, this software included the g test, which tests the notion that stable populations are reflected by highly variable variances of allele sizes among

loci, while in an expanding population this variance is more equal. Both tests were preformed here, although Luikart *et al.* (1998) demonstrated that the g test was the more powerful of the two.

Gene flow patterns between sample locations

The number of migrants between the different sample locations was estimated using Slatkin's (1985) private allele method which is incorporated in the GENEPOP 4.0 (Raymond and Rousset 1995). This calculation assumes an approximately equilibrium distribution of allele frequencies among the demes comprising a population (Barton and Slatkin 1986). Most coalescent computer programs, developed to calculate gene flow between populations and effective population size, assume stable (sub) populations over time (Kuhner 2008). As the Dutch Black-tailed Godwit population is believed to have been rather variable in size over the last 100 years (Beintema *et al.* 1995, Schekkerman *et al.* 2008, Schroeder *et al.* 2009), we refrained from using these programs. Nevertheless, there are programs such as IMa2 (Hey and Nielson 2007) that allow testing of migration rates between different locations in populations with a probably unstable subpopulation structure over time. However, this program uses a tree string as a backbone to make coalescent inferences. Unfortunately, there were several uncertainties in our data in constructing such a tree correctly and as such this program was not used further. To explore dispersal limitation issues due to the confounding effect of geographic distance a Mantel test (normally transformed and log transformed) with 9999 permutations was preformed using GENALEX 6.2 (Peakall and Smouse 2006) which calculates the correlation between a genetic and a geographic distance matrix (Smouse and Long 1992, Smouse *et al.* 1986).

Results

Genetic marker validation

A total of 140 birds from 9 different breeding locations were genotyped. All 12 loci amplified no more that two alleles per individual. All loci were polymorphic with 4 to 15 alleles per locus. We detected 126 different alleles.

Some loci in some locations exhibited significant deviations from Hardy-Weinberg. However, none of these values remained significantly different from zero after sequential Bonferroni correction. After Bonferroni correction no linkage-disequilibrium was found between any of the loci in any of the locations. The mean genotyping error, the averaged difference between the 1st and 2nd and 1st and 3rd time of scoring, was 1.5%. MICRO-CHECKER showed no presence of null alleles at any of the sample locations or loci.

Genetic diversity and F_{IS} and population structure analysis

The mean number of alleles, absolute number of alleles, allelic richness, Ho, He, F_{IS}, and private alleles, per sample location are assembled in Table 1. There was no relationship between genetic diversity values (Table 1) and sample location. F_{IS} was not significantly different from zero in any location. Subsequently, the differences in allelic richness (N =9, P = 0.079) and F_{IS} (N = 9, P = 0.866) among sample locations were not significant.

Table 1. Number of samples (N), mean number of alleles (N_a), absolute number of alleles (A), allelic richness (A_R), Observed heterozygosity (H_o), Expected heterozygosity (H_e), inbreeding coefficient (F_{IS}) and number of private alleles (P_a), per sample location (Figure 1) using 12 microsatellites.

Sample location	N	N_a	A	A_R	Ho	He	F_{IS}	P_a
ZWF	38	8	96	5.134	0.686	0.682	-0.024	5
EAV	24	7.5	90	4.987	0.628	0.674	0.053	4
ZOE	11	6	72	5.133	0.697	0.689	-0.012	1
IDZ	18	7.1	85	5.259	0.685	0.689	0.005	1
MID	11	5.8	70	5.094	0.694	0.695	-0.018	1
NOR	7	5.3	64	5.333	0.715	0.722	0.011	1
VIJF	10	6.2	74	5.351	0.683	0.679	-0.006	1
UIT	11	5.8	69	4.944	0.616	0.647	0.002	0
OVE	10	5.4	65	4.794	0.643	0.689	0.050	0
Average	15.0	6.3	76.0	5.115	0.671	0.685	0.007	1.6

Nearly all pairwise F_{ST} values between locations were not significantly different from zero, except for those between ZWF and VIJF (Table 2). However, after Bonferroni correction this significance did not hold. AMOVA calculations showed no significance for any of the calculated variances (0% V_a = -0.0011 P = 1.000 ± 0.000, 0.3% V_b = 0.004 P = 0.37793 ± 0.00353 and 99.7% V_c= 0.004 P = 0.37903 ± 0.00358).

Structure analyses indicated that the most likely value for the amount of genetic clusters (K) was K = 1. Using the method as described by Evanno *et al.* (2005) and plotting delta K did not result in a 'plateau' and as such it was not clear what value for K was the most likely. Maximum average log likelihood Ln P(P|K) values plotted against number of inferred clusters (K) demonstrated that K = 1 best fit the data (Figure 2), as the highest log likelihood was obtained with K = 1.

Table 2. Pairwise F_{ST}, on the left side of the table, and according P values, on the right side of the table, are given. P values smaller than 0.05, are indicated with *.

	ZWF	EAV	ZOE	IDZ	MID	NOR	VIJF	UIT	OVE
ZWF		0.085±0.002	0.592±0.003	0.545±0.003	0.709±0.003	0.348±0.003	0.022±0.001*	0.947±0.0015	0.310±0.003
EAV	0.005		0.691±0.003	0.252±0.003	0.451±0.004	0.743±0.003	0.051±0.002	0.607±0.003	0.278±0.000
ZOE	-0.002	-0.003		0.554±0.004	0.901±0.002	0.525±0.003	0.173±0.003	0.849±0.003	0.630±0.003
IDZ	-0.001	0.004	-0.002		0.528±0.003	0.381±0.004	0.308±0.003	0.973±0.001	0.453±0.004
MID	-0.004	0.001	-0.011	-0.001		0.684±0.003	0.961±0.001	0.959±0.001	0.504±0.004
NOR	0.002	-0.005	-0.002	0.003	-0.007		0.814±0.003	0.142±0.002	0.670±0.003
VIJF	0.013	0.015	0.008	0.003	-0.015	-0.010		0.528±0.003	0.329±0.004
UIT	-0.009	-0.001	-0.010	-0.013	-0.017	0.013	-0.001		0.885±0.002
OVE	0.003	0.006	-0.004	0.001	0.000	-0.004	0.005	-0.011	

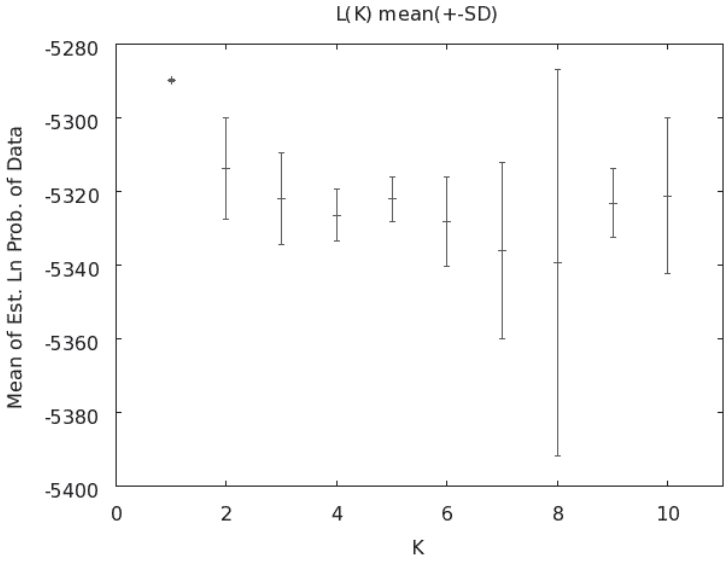

Figure 2. Mean log likelihood Ln P(X|K) as a function of the number of genetic clusters (K) averaged over 5 consecutive STRUCTURE runs for each K (error bars indicate one standard deviation).

Kgtests (Banlin 2007) indicated no population expansion in the dataset. The k test revealed that 9 out of 12 loci had a negative kurtosis which was almost significant (P = 0.06). The g test estimated that the variance of the allele sizes was 8.01. According to the fifth-percentile cutoff table given by Reich *et al.* (1999) this value was not significant.

Gene flow patterns between sample locations

Mantel tests detected no significant correlation between genetic distance and either measure of geographic distance (P = 0.157 normally transformed and log transformed P = 0.448). The number of migrants (Nm) per generation (Table 3) showed a range from 4.96 (between ZWF and EAV) to 1.50 (between NOR and OVE). The average number of migrants per generation among the Dutch breeding locations, and corrected for sample size, was 2.79.

Table 3. Number of migrants per generation between 2 sample locations.

	ZWF	EAV	ZOE	IDZ	MID	NOR	VIJF	UIT	OVE
ZWF		4.96	3.93	4.74	3.17	2.35	3.22	3.99	2.66
EAV	4.96		3.41	3.18	3.65	2.36	2.48	4.12	2.49
ZOE	3.93	3.41		2.63	2.28	1.98	1.92	2.68	2.35
IDZ	4.74	3.18	2.63		2.56	2.12	4.10	3.65	2.49
MID	3.17	3.65	2.28	2.56		1.76	2.10	2.50	2.09
NOR	2.35	2.36	1.98	2.12	1.76		1.79	1.82	1.50
VIJF	3.22	2.48	1.92	4.10	2.10	1.79		2.70	2.88
UIT	3.99	4.12	2.68	3.65	2.50	1.82	2.70		1.92
OVE	2.66	2.49	2.35	2.49	2.09	1.50	2.88	1.92	

Discussion

We demonstrated an absence of genetic structure among nine Dutch Black-tailed Godwit breeding locations. Does this lack of genetic structure reflect limited power due to an insufficient number of microsatellites (Barnett *et al.* 2008)? We suggest this not to be the case as several studies have shown genetic structure using seven or even fewer microsatellites (Davis *et al.* 2006, Rönkä *et al.* 2008). Additionally, several studies demonstrated the lack of genetic structure using as many as 13 (Barnett *et al.* 2008) and 29 microsatellite markers (Van Treuren *et al.* 1999). Furthermore, the microsatellites used did not exhibit Hardy-Weinberg and linkage disequilibrium and showed no null allele problem. All the loci used were polymorphic with ranges between 4 and 15 alleles and a total of 126 alleles in the entire dataset. According to Ryman *et al.* (2006) these values of allelic range and total amount of alleles in combination with a sample size of 140 individuals and 12 microsatellites used, should be sufficient to detect genetic structure. As such it seems very unlikely that absence of genetic structure with the Dutch Black-tailed Godwit population can be appointed to insensitivity or amount of the microsatellites used.

Nevertheless, genetic structure can be influenced by the dispersal abilities and the extent of habitat fragmentation of the species under study. The detection of dispersal abilities and habitat fragmentation depend on the scale of the study. As such the detection of genetic structure depends to a large degree on the scale of the study as well (Barnett *et al.* 2008). In studies on other bird species (Temple *et al.* 2006, Woxvold *et al.* 2006), genetic structure was evident using only six microsatellites on much smaller scales (longest distance between locations 4 km and 8 km) compared to the spatial scale (a maximum of 134 km between sites) in this study. This demonstrates that even on very small spatial scales, in some birds, genetic structure can be detected. Obviously, this depends on the birdspecies under study as well as the landscape it lives in.

The multi-locus microsatellite data presented here suggest the most likely explanation for the lack of genetic structure in the Dutch Black-tailed Godwit is that breeding areas in The Netherlands comprise one panmictic unit. Gene flow estimates demonstrated an overall migration rate of three individuals per generation among Dutch breeding locations. According to both Mills and Allendorf (1996) and Slatkin (1985) this rate should be enough to minimize genetic differentiation. Subsequently, results of the Mantel test demonstrated no isolation by distance that would indicate restrictions on gene flow. This shows that dispersal movements have taken place well beyond the 18 km range, as far as 134 km, which demonstrates the high breeding mobility capabilities of this species. Groen (1993) and van den Brink *et al.* (2008) have most likely underestimated the dispersal distance of this species. This is supported by unpublished observations of Kentie *et al.* which indicate that adult birds seek out new breeding sites up to 10 km from the previous breeding site. Still, the most probable explanation for these findings is natal dispersal.

Bayesian analysis in STRUCTURE showed that all individual Black-tailed Godwits could most likely be assigned to one genetic cluster. This was supported by pairwise F_{ST} calculations which demonstrated little (ranging from -0.0168 to 0.01337) or no significant differentiation between locations.

Additionally, none of the F_{IS} values were significantly different from zero for any of the locations, indicating no inbreeding . Subsequently, AMOVA showed that more than 99% of the molecular variation was found across all individuals while an insignificant proportion (0.3%) was attributable to variation between individuals from different locations.

Interestingly, Schroeder *et al.* (2010) showed the presence of a polymorphism in the CHD1-Z gene. The rare polymorphism (Z*, 14% of population) appeared to be associated with fitness advantages and only occurred in Black-tailed Godwit breeding in reserves managed as meadowbird habitat. Although there still is the problem of statistical power (33 individuals sampled outside meadowbird reserves and 251 birds in reserves), the fitness correlates indicated that this genetic marker was non-selectively neutral and thereby not a suitable marker for evaluating neutral population structuring (Schroeder *et al.* 2010). Wolf *et al.* (2010) suggested that markers that are linked to genes under expression evolve much faster than do non-coding genes. This may in turn have resulted in incipient population structuring measurable with the appropriate non-selective neutral marker, whilst it has not lead to it being measurable using neutral markers.

The level of genetic diversity in the Dutch Black-tailed Godwit in this study is higher (average of 6.3 alleles per locus) compared to that reported by Höglund *et al.* (2009) who found rather low genetic diversity (1 haplotype) within the Dutch Black-tailed Godwit (*Limosa limosa limosa*) using mitochondrial DNA. However, the marker used in this study amplifies the second domain of the control region a part that is highly conserved (Höglund *et al.* 2009) and might therefore be less suitable for detection of genetic variation within the Dutch Black-tailed Godwit. The genetic diversity within the Dutch Black-tailed Godwit was therefore likely underestimated in this study. Furthermore, most Dutch samples came from the Western part of The Netherlands while 50% of the population resides in the northern part of the country, possibly holding a significant amount of the total population genetic variation.

Interestingly, using mtDNA from four different Dutch Black-tailed Godwit breeding sites Höglund *et al.* (2009) did not detect any genetic structure either. This result together with the results demonstrated here would indicate that all locations are affected by long term panmixis or that possible gene flow between different breeding areas ceased too recently for both marker types to be detectable (Zink and Barrowclough 2008).

All together it appears that the Dutch Black-tailed Godwit population is not confronted with immediate genetic threats and we argue that according to these data the Dutch Black-tailed Godwit should be managed as one panmictic unit.

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References

Barnett JR, Ruiz-Gutierrez V, Coulon A, Lovette IJ (2008) Weak genetic structuring indicates ongoing gene flow across White-ruffed Manakin (*Corapipo Altera*) populations in a highly fragmented Costa Rica landscape. Conservation Genetics, 9, 1403-1412.

Barton NH, Slatkin M (1986) A Quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity, 56, 409-415.

Beintema AJ, Beintema-Hietbrink RJ, Muskens GJDM (1985) A shift in the timing of breeding in meadow birds. Ardea, 73, 83-89.

Beintema A, Moedt O, Ellinger D (1995) Ecologische Atlas van de Nederlandse Weidevogels. Schuyt & Co, Haarlem.

Benton TG, Bryant DM, Cole L, Crick HOP (2002) Linking agricultural practice to insect and birdpopulations: a historical study over three decades. Journal of Applied Ecology, 39, 673-687.

Bijlsma RG, Hustings F, Camphuysen CJ (2001) Algemene en schaarse vogels van Nederland (Avifauna van Nederland 2). Haarlem/Utrecht: GMB Uitgeverij/KNNV Uitgeverij.

Bilgin R (2007) Kgtests: a smiple Excel Macro program to detect signatures of population expansion using microsatellites. Molecular Ecology Notes, 7, 416-417.

BirdLife International (2009) Species factsheet: *Limosa limosa*. Downloaded from http://www.birdlife.org on 29/1/2010.

Birdlife International (2004). Birds in Europe: population estimates, trends and conservation status. Cambridge, UK.

Chamberlain DE, Fuller RJ, Bunce RGH, Duckworth JC, Shrubb M (2000) Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. Journal of Applied Ecology, 37, 771-788.

Davis LA, Roalson EH, Cornell KL, McClanahan KD, Webster MS (2006) Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. Molecular Ecology, 15, 2141-2152.

Dent EA (2009) STRUCTURE HARVESTER version 0.56.3 http://taylor0.biology.ucla.edu/struct_harvest/.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14, 2611-2620.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

Glabutz JC (2004) CONVERT: a user friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes, 4, 309-310.

Groen NM (1993) Breeding site tenacity and natal philopatry in the Black-tailed Godwit. Ardea, 81, 107-113.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity, 86, 485-486.

Haverschmidt F (1963) The black-tailed godwit. E.J. Brill. Leiden

Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. PNAS, 104, 2785-2790.

Höglund J, Johansson T, Beintema A, Schekkerman H (2009) Phylogeography of the Black-tailed Godwit *Limosa limosa*: substructuring revealed by mtDNA control region sequences. Journal of Ornithology, 150, 45-53.

Höglund J (2009) Evolutionary Conservation Genetics. Oxford university press, Oxford, New York.

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. Ecology, 52, 577-586.

Kuhner MK (2009) Coalescent genealogy samplers: windows into population history. Trends Ecology and Evolution, 24, 86-92.

Liebezeit JR, Smith PA, Lanctot RB, Schekkerman H, Tulp I, Kendall SJ, Tracy DM, Rodriques RJ, Meltofte H, Robinson JA, Gratto-Trevor C, McCaffery BJ, Morse J, Zack SW (2007) Assessing the development of shorebird eggs using the flotation method: Species-specific and generalized regression models. Condor, 109, 32-47

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology, 7, 963–974.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. Conservation Biology, 10, 1509-1518.

Mulder T (1972) De grutto (*Limosa limosa* (L)) in Nederland: aantallen, verspreiding terreinkeuze, trek en overwintering. Bureau van de KNNV, Hoogwoud.

Peakal R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6, 288-295.

Pritchard JK, Stphens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Qiagen (2003) DNeasy Tissue Handbook. Protocol for isolation of total DNA from animal tissues. pp.18-20, QIAGEN. Valencia, California, USA.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Reich DE, Feldman MW, Goldstein DB (1999) Statistical properties of two tests that use multilocus data sets to detect population expansions. Molecular Biology and Evolution, 16, 453-466.

Rice WR (1989) Analysing tables of statistical tests. Evolution, 43, 223-225

Richardson DS, Jury FL, Blaakmeer K, Komdeur J, Burke T (2001) Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). Molecular Ecology, 10, 2263-2273.

Rönkä A, Kvist L, Karvonen J, Koivula K, Pakanen VM, Schamel D, Tracy DM (2008) Population genetic structure in the Temminck's stint *Calidris temminckii*, with an emphasis on Fennoscandian populations. Conservation Genetis, 9, 29-37.

Ryman N, Palm S, Andre C, Carvalho GR, Dahlgren TG, Jorde PR, Laikre L, Larsson LC, Palme A, Ruzzante DE (2006) Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology, 15, 2031-2045.

Schekkerman H, Teunissen WA, Oosterveld E (2009) Morality of Black-tailed Godwit *Limosa limosa* and Northern Lapwing *Vanallus vanellus* chicks in wet grasslands: influence of predation and agriculture. Journal of Ornithology, 150, 133-145

Schekkerman H, Teunissen WA, Oosterveld E (2008) The effect of 'mosaic management' on the demography of black-tailed godwit *Limosa limosa* on farmland. Journal of Applied Ecology, 45, 1067-1075.

Schröder J, Hinsch M, Hooimeijer JCE, Piersma T (2009). Faillissement dreigt voor Nederlands weidevogelbeleid. De Levende Natuur, 110, 333-338.

Schroeder J, Kentie R, van der Velde M, Hooijmeijer JCEW, Both C, Haddrath O, Baker AJ, Piersma T (2010) Linking intronic polymorphism on the CHD1-Z gene with fitness correlates in Black-tailed Godwits *Limosa l. limosa*. Ibis, 152, 368-377.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, 236, 787-792.

Slatkin M (1985) Gene flow in natural popualtions. Annual review of Ecology and systematic, 16, 393-430.

Smouse PE, Long JC (1992) Matrix correlation analysis in anthropology and genetics. Yearbook Physiological Anthropology, 35, 187-213.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology, 35, 627-632.

Stoate C, Báldi A, Beja P, Boatman ND, Herzon I, van Doorn A, de Snoo GR, Rakosy L, Ramwell C (2009) Ecological impacts of early 21st century agricultural change in Europe – A review. Journal of Environmental Management, 91, 22-46.

Temple HJ, Hoffman JI, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher, *Ramphocinclus brachyurus*. Molecular Ecology, 15, 3449-3458.

Teunissen W, Soldaat L (2005) Indexen en trends van een aantal weidevogelsoorten uit het Weidevogelmeetnet. Periode 1990-2004. SOVON-informatie 2005/13 SOVON Vogelonderzoek Nederland Beek-Ubbergen.

Trimbos KB, Broekman J, Kentie R, Musters CJM, de Snoo GR (2009) Using eggshell membranes as a DNA source for population genetic research. Journal of Ornithology, 150, 915-920.

van den Brink V, Schroeder J, Both C, Lourenco PM, Hooijmeijer JCEW, Piersma T (2008) Space use by Black-tailed Godwits *Limosa limosa limosa* during settlement at a previous or a new nest location. Bird Study, 55, 188-193.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes, 4, 535–538.

van Treuren R, Bijlsma R, Tinbergen JM, Heg D, van de Zande L (1999) Genetic analysis of the population structure of socially organized Oystercatchers (*Haemotoopus ostralegus*) using microsatellites. Molecular Ecology, 8, 181-187.

van Turnhout CAM, Foppen RPB, Leuven RSEW, Siepel H, Esselink H (2007) Scale-dependent homogenization: Changes in breeding bird diversity in the Netherlands over a 25-year period. Biological Conservation, 134, 505-516.

Verkuil YI, Trimbos K, Haddrath O, Baker AJ (2009) Characterization of polymorphic microsatellite DNA markers in the black-tailed godwit (*Limosa limosa: Aves*). Molecular Ecology Resoures, 9, 1415-1418.

Vucetich JA, Waite TA (2001) Migration and inbreeding: the importance of recipient population size for genetic management. Conservation Genetics, 2, 167-171.

Wolf JBW, Bayer T, Haubold B, Schilhabel M, Rosenstiel P, Tautz D (2010) Nucleotide divergence vs. gene expression differentiation: comparative transcriptome sequencing in natural isolates from the carrion crow and its hybrid zone with the hooded crow. Molecular Ecology, 19, 162-175.

Woxvold IA, Adcock GJ, Mulder RA (2006) Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds. Molecular Ecology, 15, 3139-3146.

Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology, 17, 2107-2121.

Zwarts L, Bijlsma RG, van der Kamp J, Wymenga E (2009) Living on the edge. Wetlands and birds in a changing Sahel. KNNV Publishing Zeist.