

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/271913374>

Fluctuating asymmetry and inbreeding in Scandinavian gray wolves (*Canis lupus*)

Article in ACTA THERIOLOGICA · July 2014

DOI: 10.1007/s13364-014-0174-0

CITATIONS

6

READS

299

2 authors, including:



Øystein Wiig

Natural History Museum, Norway

306 PUBLICATIONS 12,699 CITATIONS

SEE PROFILE

Fluctuating asymmetry and inbreeding in Scandinavian gray wolves (*Canis lupus*)

Øystein Wiig · Lutz Bachmann

Received: 17 October 2013 / Accepted: 21 January 2014 / Published online: 4 February 2014
© Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland 2014

Abstract We investigated fluctuating asymmetry in 13 traits of the skulls and jaws of historical and contemporary populations of Scandinavian gray wolves (*Canis lupus*). We hypothesized that there is a higher level of fluctuating asymmetry in the inbred contemporary population than in the historical population. Our analyses did not detect any difference in the level of fluctuating asymmetry as predicted. We propose different explanation for this lack of change in fluctuating asymmetry. It is assumed that a large number of studies have failed to find a positive correlation between the level of genetic stress and developmental instability and have therefore never been published, which hampers a good understanding of fluctuating asymmetry as an indicator of developmental instability. The current study is thus important in this context. The gray wolf population in Scandinavia is characterized by an extreme bottleneck followed by two and a half decades of strong inbreeding, but no associated change in fluctuating asymmetry is detected.

Keywords Carnivora · Gray wolf · Scandinavia · Skull · Fluctuating asymmetry · Inbreeding

Introduction

The gray wolf (*Canis lupus*) has a widespread distribution in North America and Eurasia (Nowak 2003). In the late 19th century, the wolf was still common in large areas in Fennoscandia, Russia, and the Baltic states (Pulliainen 1985). However, wolves became virtually extinct in Scandinavia by

the end of the 1960s due to extensive hunting (Flagstad et al. 2003). The wolves were legally protected in 1966 in Sweden and in 1972 in Norway (Wabakken et al. 2001). In the early 1980s, at least two wolves immigrated to southern Scandinavia probably from Russia or Finland and gave birth to a litter in 1983 (Wabakken et al. 2001; Vilà et al. 2003). Since then, wolves have regularly reproduced in southern Scandinavia. This led to an increased number and expanded range (Wabakken et al. 2001), and today, there are between 250 and 300 wolves in southern Scandinavia (Wabakken et al. 2011).

Flagstad et al. (2003) examined the genetic variability of Scandinavian wolves from 1829 to 1979 and detected a decreasing level of variability until the mid 1900s. Immigration from eastern populations after 1940 contributed to an increase in the level of genetic variation thereafter. Nevertheless, the genetic diversity of gray wolves was low the first years after the recolonization in the early 1980s but increased again after the arrival of a single new male immigrant in 1991 (Vilà et al. 2003). Thus, the history of the current Scandinavian wolves is characterized by an extreme bottleneck followed by two and a half decades of strong inbreeding (Hagenblad et al. 2009). Accordingly, inbreeding depression has been documented (Liberg et al. 2005). Also, Räikkönen et al. (2006) investigated the morphology of vertebral malformations and their frequency among historical and contemporary Scandinavian wolves and an eastern reference population. The frequency of lumbosacral transitional vertebrae was significantly higher in the contemporary Scandinavian wolves than in an eastern reference population, and no such segments were found in the historical group. The higher occurrence in the contemporary group was considered an indication of inbreeding depression.

Fluctuating asymmetry (FA) describes subtle random differences in trait expression in bilaterally symmetrical structures (Palmer and Strobeck 2003a). If the two homologous sides in a bilateral organism differ significantly in growth or form, they are considered asymmetric (Palmer and Strobeck

Communicated by: Cino Pertoldi

Ø. Wiig (✉) · L. Bachmann
Natural History Museum, University of Oslo,
PO Box 1172, Blindern, 0318 Oslo, Norway
e-mail: oystein.wiig@nhm.uio.no

2003a). FA is an accepted measure for developmental instability, which is attributed to an imperfect expression of developmental design due to disturbances (i.e., environmental and genetic stressors) during development (Palmer and Strobeck 2003a). Some case studies have shown an increased level of FA following a population bottleneck. Hoelzel et al. (2002), for example, found an increased FA in a northern elephant seal (*Mirounga angustirostris*) population following a severe decline in population size at the end of the 19th century, and Lovatt and Hoelzel (2011) found similar results for reindeer (*Rangifer tarandus*) in South Georgia. The aim of the present study was to investigate FA in the skulls of historical and contemporary populations of Scandinavian gray wolves that recently have gone through a genetic bottleneck. We hypothesized that there would be a higher level of FA in the inbred contemporary population than in the historical population.

Materials and methods

A total of 118 gray wolf skulls from the Natural History Museum, University of Oslo (NHM), Bergen Museum, University of Bergen (BM), and Naturhistoriska Riksmuseet, Stockholm (NHR) were examined (Table 1). The skulls had been collected from wild living wolves in Scandinavia in the period between 1883 and 2005.

The individual age in months was determined for most specimens from incremental growth layers in the cementum of a canine tooth (Landon et al. 1998) and an assumed date of birth. Pups of Scandinavian gray wolves are born in late April and May (Wabakken et al. 2013), and we used 1 May in this analysis. The age determination was done by the Matson Labaoratory (LLC, Milltown, Montana, USA) and by the Norwegian Institute of Nature Research (Trondheim, Norway). When tooth ages were not available, a visual age determination was done on the skulls during measurement (according to Gibson et al. 2000). Most of the body growth in gray wolves is completed by the end of the first year (Peters 1993). Skulls determined as younger than 1 year were excluded from the analyses in order to include only skulls where most of the growth were completed. In order to investigate age

Table 1 Sex and age distribution of skulls of Scandinavian gray wolves from historic (pre 1980) and contemporary (after 1980) populations used for studying fluctuating asymmetry

Sex	Historic population		Contemporary population	
	Subadults	Adults	Subadults	Adults
Unknown	3 (1)	12 (6)	3 (2)	1 (1)
Males	2 (3)	15 (8)	20 (2)	27 (13)
Females	3 (3)	5 (2)	11 (0)	16 (5)
Total	40 (23)		78 (23)	

variation, skulls 1 year and older were divided into subadults (<2 years) and adults (>2 years).

Thirteen bilateral metric traits (Table 2, Fig. 1) were measured to the nearest 0.01 mm on the right and left sides of each skull using digital calipers (Mitutoyo, Mitutoyo Corporation, Japan).

In order to assess measurement error (ME) (Palmer and Strobeck 1986), replicate measurements were taken on the skulls located at NHM and BM ($N=46$) (Table 1). The number is within the minimum of 30 recommended by Palmer (1994). The traits were measured twice for each skull, always by the same person but never on the same day. If exact measurements could not be taken because of damage of the skull, this was indicated by “missing value” in the data matrix.

To estimate whether FA was significantly greater than ME, a two-way mixed model ANOVA (Palmer and Strobeck 1986; Bechshøft et al. 2008a) was performed on the subsample with the replicate measurements. The replicate measurements were used as the dependent variable with individual as random and side as fixed factors for each of the traits. Descriptors of FA

Table 2 Bilateral morphological traits measured on the skulls of Scandinavian gray wolves. Nomenclature according to von den Driesch (1976) and Bechshøft et al. (2008a, b)

Trait	Description
Skull	
CBL	Condylbasal length. The maximal distance between the anterior margin of the alveoli of the 1th incisor to the anterior margin of the occipital condyle.
OPF	Maximal distance between the <i>opistocranium</i> and the postorbital process of the frontal bone.
POH	Postorbital height. The minimal distance between the postorbital process of the frontal bone and the frontal process of the zygomatic arch.
PL	Palatal length. The distance from the posterior border of the incisive foramen to staphylion.
P4-M2	Length from the anterior margin of the 4th premolar to the posterior margin of the 2nd molar.
P1-M2	Length from the anterior of the alveoli of the 1th premolar to the posterior of the alveoli of the 2nd molar.
I1-M2	Length from the anterior of the alveoli of the 1th incisor to the posterior of the alveoli of the 2nd molar.
P4L	Length of the 4th premolar.
Lower jaw	
ML	Mandible length. The maximal distance from the anterior margin of the mandibular symphysis and the posterior margin of the angular process.
MH	Mandible height. The maximal distance between the margin of the angular process and the coronoid process.
p1-m3	Length from the anterior of the alveoli of the 1th premolar to the posterior of the alveoli of the 3th molar.
i1-m3	Length from the anterior of the alveoli of the 1th incisor to the posterior of the alveoli of the 3th molar.
m1L	Length of the 1th molar.

Fig. 1 Bilateral morphological traits measured on the skull of Scandinavian gray wolves. Definitions of traits are given in Table 2



and ME were calculated and used according to Palmer (1994), Palmer and Strobeck (2003a, b), and Bechshøft et al. (2008b). The MS_{si} (mean squares of the sides \times individuals' interaction) and MS_{error} (mean squares of the variance of the repeat measurements) were used to estimate FA excluding ME in mm (FA10a), MS_{error} as % of MS_{si} (ME3), repeatability (ME5), average difference between the repeated measurements in mm (ME1), FA including ME (FA4a).

A Shapiro–Wilk test was used to test the significance of any departure from normality of differences between right and left (R–L) sides. The presence of directional asymmetry of (R–L) was tested by a two-tailed one-sample *t*-test against a mean of zero (Palmer 1994). Size dependency for each trait was tested by Spearman's correlation coefficient between $(|R-L|)$ and $((R+L)/2)$ (Palmer 1994).

A one-way ANOVA with $(|R-L|)$ as dependent variable, and sex or age groups as independent variables, was performed for each trait to investigate if a difference in FA was attributable to age and/or sex. Possible change over time in FA was investigated for each trait by a one-way ANOVA with $(|R-L|)$ as dependent variable and time period (historic and contemporary samples) as fixed variable. FA over time was also analyzed by estimating Pearson's correlation between $(|R-L|)$ and year of birth for each trait. The specimen-wide level of FA was

estimated from $(|\ln(R)-\ln(L)|)$ pooled over several selected traits (Palmer and Strobeck 2003a; Bechshøft et al. 2008b).

All statistical analyses were performed using SPSS version 16.0. The significance level was set to $\alpha=0.05$, but Bonferroni corrections for multiple tests were also applied throughout the study (Palmer 1994).

Results

ME was found to be smaller than FA for all 13 traits (Table 3). The FA10a, i.e., FA corrected for ME, ranged from 0.15 to 1.34 mm. Measurement repeatability of the traits (ME5) ranged from 79 to 97 %, except from traits MH and m1L, which showed a low repeatability (38 and 45 %, respectively). The error variance contributed from 1.77 to 11.99 % of total variance between sides (ME3), except for traits MH and m1L with 45.02 and 38.33 %, respectively.

Traits P4-M2, p1-m3, i1-m3, P4L, and m1L had a distribution significantly different from normal (Table 4). Trait POH and MH showed directional asymmetry, and none of the traits was dependent on size (Table 4). The seven traits POH, P4-M2, MH, p1-m3, i1-m3, P4L, and m1L were accordingly excluded from further analyses.

Table 3 Results of the two-way mixed model ANOVA (sides as fixed factor and individuals as random factor) for 13 metric traits measured twice on the skulls of Scandinavian gray wolves ($N=46$)

	CBL	OPF	POH	PL	P4-M2	P1-M2	Trait I1-M2	P4L	ML	MH	p1-m3	i1-m3	m1L
MS_{si}^a	0.386	1.009	0.999	0.182	0.282	1.15	0.719	0.207	1.45	0.884	2.889	1.253	0.06
MS_{se}^b	0.036	0.121	0.074	0.017	0.014	0.097	0.058	0.005	0.059	0.398	0.051	0.056	0.023
Df^c	38	42	42	43	43	45	45	42	44	44	43	43	41
F^d	10.78	8.365	13.58	10.73	20.1	11.83	12.49	38.58	24.77	2.221	56.62	22.3	2.635
P^e	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FA10a ^f	0.472	0.752	0.767	0.324	0.413	0.819	0.649	0.359	0.941	0.556	1.344	0.873	0.153
ME3 ^g	9.326	11.99	7.407	9.341	4.965	8.435	8.067	2.415	4.069	45.02	1.765	4.469	38.33
ME5 ^h	0.829	0.786	0.862	0.829	0.905	0.844	0.851	0.953	0.922	0.379	0.965	0.914	0.446
FA4a	0.495	0.802	0.797	0.340	0.666	0.856	0.677	0.363	0.961	0.750	1.356	0.893	0.196
ME1 ⁱ	0.151	0.278	0.217	0.104	0.094	0.249	0.192	0.056	0.194	0.503	0.18	0.189	0.121

Descriptors of FA (fluctuating asymmetry) and ME (measurement error) were calculated and used according to (Palmer and Strobeck 2003b): MS_{si} (mean squares of the sides \times individuals' interaction), MS_{error} (mean squares of the variance of the repeat measurements (error)), FA10a (FA excluding ME, in mm), ME3 (MS_{error} as % of MS_{si}), ME5 (repeatability), and ME1 (average difference between the repeated measurements, in mm)

^a Mean squares of the sides \times individuals' interaction of the two-way mixed model ANOVA

^b Mean squares of the variance of the repeated measurements (error)

^c Degrees of freedom for the different traits as depends on number of skulls measured at each trait

^d *F*-values obtained from the two-way mixed model ANOVA

^e Significant values (*p*-values) of whether measurement error is significantly lower than FA ($|R-L|$)

^f FA10a = $0.798\sqrt{MS_{si}-MS_{error}}$, in mm

^g ME3: MS_{error} as % of MS_{si}

^h ME5 = $(MS_{si}-MS_{error})/(MS_{si}+(2-1)MS_{error})$

ⁱ ME1 = $0.798\sqrt{MS_{error}}$

Table 4 Tests for normality (Shapiro–Wilk: *W*), directional asymmetry (one-sample *t*-test) and size dependency of differences between sides (Spearman's correlation) in 13 bilateral traits measured on the skulls ofScandinavian gray wolves (*n*=118). Not all traits on all skulls were available for measurement

Trait	Shapiro–Wilk			One-sample <i>t</i> -test		Spearman's correlation	
	<i>n</i>	<i>W</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>r</i>	<i>p</i>
CBL	102	0.982	0.190	−2.291	0.024	−0.002	0.993
OPF	96	0.988	0.536	0.590	0.556	0.099	0.339
POH	105	0.997	0.997	3.076	0.003*	−0.159	0.105
PL	115	0.992	0.749	−1.805	0.074	−0.047	0.620
P4-M2	115	0.948	0.000*	−1.154	0.251	0.069	0.461
P1-M2	118	0.994	0.866	−0.481	0.631	−0.036	0.699
I1-M2	117	0.989	0.442	1.712	0.090	0.049	0.601
P4L	109	0.852	0.000*	−0.684	0.496	−0.210	0.028
ML	108	0.989	0.489	1.420	0.159	0.033	0.736
MH	109	0.995	0.959	−2.947	0.004*	0.019	0.843
p1-m3	110	0.854	0.000*	−1.456	0.148	−0.211	0.027
i1-m3	109	0.994	0.000*	−1.854	0.066	−0.069	0.478
m1L	101	0.912	0.000*	0.191	0.849	−0.029	0.773

* Statistically significant at the $\alpha=0.05$ level after a sequential Bonferroni correction for multiple tests

The one-way ANOVA showed no significant differences in FA for sex or age groups after Bonferroni corrections for multiple tests for any of the remaining six traits (OPF, PL, P1-M2, I1-M2, ML, and CBL) (Table 5), and all groups were thus combined. The results of the one-way ANOVA with FA ($|R-L|$) as dependent variable and historic and contemporary populations as fixed variables showed no significant differences (Table 6). No significant correlations between FA and year of birth were detected (Table 7). No correlations with the year of birth were found in the specimen-wide measure of asymmetry ($n=66$, $\rho=-0.025$, $p=0.84$). Consequently, our analyses did not detect any difference in the level of FA as predicted.

Discussion

FA is often in the order of magnitude of 1 % of trait size (Palmer 1994). In this study, FA varied between 0.8 % (OPF) and 3.4 % (POH) of trait size. In order to detect such subtle asymmetries,

the FA index must be significantly greater than the ME to justify further investigation. In this study, the FA of all traits was significantly greater than the ME.

Two traits (POH and MH) showed directional asymmetry. This may either reflect true difference in bone development between sides or be a result of handedness from the person did the measurements. MH also had a high ME and low measurement repeatability. We believe that the directional asymmetry in these two traits is due to problems connected to taking the measurements and not related to differences in bone growth. The two traits were excluded from the FA analyses as recommended by Palmer and Strobeck (2003a).

The history of the population of Scandinavian wolves is characterized by an extreme bottleneck followed by two and a half decades of strong inbreeding (e.g., Hagenblad et al. 2009). Inbreeding depression in the population has been documented genetically (Liberg et al. 2005) as well as morphologically (Räikkönen et al. 2006). Inbreeding has been reported to depress symmetry during development and measurements of

Table 5 Differences between age classes (subadults and adults) within each sex and differences between sexes within each age class in fluctuating asymmetry of six bilateral traits measured on the skulls of Scandinavian gray wolves. Level of significance at the $\alpha=0.05$ level is evaluated based on a sequential Bonferroni correction for multiple tests.

Trait	Males			Females			Subadults			Adult		
	<i>DF</i>	<i>F</i>	<i>p</i>	<i>DF</i>	<i>F</i>	<i>p</i>	<i>DF</i>	<i>F</i>	<i>p</i>	<i>DF</i>	<i>F</i>	<i>p</i>
CBL	54	0.440	0.510	30	0.047	0.831	32	1.081	0.307	52	0.267	0.608
OPF	49	0.119	0.741	25	2.018	0.168	21	2.098	0.162	53	1.349	0.251
PL	59	0.146	0.703	33	2.328	0.137	34	0.729	0.399	58	1.608	0.210
P1-M2	62	0.263	0.512	33	1.219	0.610	34	0.888	0.353	61	0.630	0.430
I1-M2	61	5.644	0.021	33	1.267	0.268	34	3.521	0.069	60	1.349	0.250
ML	58	0.076	0.784	29	0.181	0.673	31	0.009	0.923	56	0.805	0.374

Table 6 Differences between historic and contemporary populations in fluctuating asymmetry of six bilateral traits measured on the skulls of Scandinavian gray wolves

Trait	DF	F	p
CBL	100	0.067	0.796
OPF	94	0.141	0.798
PL	113	0.000	0.985
P1-M2	116	1.460	0.229
I1-M2	115	0.323	0.571
ML	106	0.079	0.780

morphological traits might be used to study inbreeding depression (e.g., Lacy and Alaks 2013). Studies of several mammalian species have shown an increased level of FA following population bottlenecks (e.g., Hoelzel et al. 2002; Lovatt and Hoelzel 2011). Lacy and Alaks (2013) pointed out that although associations between heterozygosity and FA have been reported in many species, such association is not always found and the generality of the relation between heterozygosity and FA has been questioned by several authors (e.g., Palmer and Strobeck 1986; Vøllestad et al. 1999). In the present study, we found no difference in FA between the historic and contemporary gray wolf skulls in spite of clear indications of inbreeding depression in the contemporary population. We propose different explanation for this lack of change in FA.

Bensch et al. (2006) found that heterozygosity in the Scandinavian wolf population had not dropped in parallel with the inbreeding coefficient. It was the most heterozygous wolves that were recruited into the breeding population, which indicated an apparent selection against homozygous individuals. So maybe the level of heterozygosity was not so low as to cause detectable increased levels in FA in the traits investigated. However, Hagenblad et al. (2009) did not find evidence for a general selection for heterozygotes in the population.

Leamy et al. (2001) found an impact on skeletal size measurements but no effect on FA, in a population of wild house mice (*Mus musculus*) that displayed large inbreeding depression. Similar results were obtained by Lacy and Alaks (2013) in a study of *Peromyscus polionotus* mice. These authors concluded that morphological asymmetry may not be a sensitive measure of genetic stress although effects of inbreeding on FA can be demonstrated when sample sizes are large. They had to measure eight bilateral traits in 896

individuals in order to detect significant inbreeding depression in FA in just one of the measured traits. The sample size in the present study was relatively low ($n=118$, Table 1), but comparable to that of Hoelzel et al. (2002) ($n=85$) and Lovatt and Hoelzel (2011) ($n=111$) who documented increased FA after genetic bottlenecks.

The relationship between FA and stress is expected to be weak and variable and dependent of the characters assessed (Leung and Forbes 1997; Clarke 1998). Traits might also be taxon-specific (Lovatt and Hoelzel 2011). So even though the traits applied in the present study have revealed FA in studies of other species, the real degree of developmental instability in the Scandinavian wolves may thus not have been captured by the traits analyzed.

The association between FA and heterozygosity may depend upon the amount of stress experienced by the organism during development (Palmer 1994). If this is so, we should expect the association between asymmetry and heterozygosity to be more important in stressful than in benign environments (Vøllestad et al. 1999). Several different kinds of stressors are recognized to have effects on FA such as chemicals, polluted habitats, extreme temperatures, audiogenic stress, food deficiency, social stress, inbreeding, sexual selection, diseases, and parasites (see e.g., Pertoldi et al. 1997; Bechshøft et al. 2008a); Pertoldi et al. (2000) showed that the Eurasian otter (*Lutra lutra*) has a low canalization of skull traits and therefore have a high phenotypic plasticity. Based on the Baldwin effect, they further anticipated high FA in populations that are undergoing a change of the reaction norm or are living under sub-optimal conditions and low FA in populations that are not undergoing directional changes and are not subject to environmental stress. Pertoldi et al. (2006) pointed out that the inverse relationship between heterozygosity and developmental instability may only become apparent in certain ecological/ or population contexts, possibly because it is concealed by various exogenic or endogenic factors. Mattisson et al. (2013) studied home range sizes of Scandinavian wolves in the period 1999–2011 and found no effect of population size or intraspecific competition on home range sizes. They suggested that the population is still in a recolonization phase and has not yet reached the threshold where density has become a limiting factor on space use. So it could be that the lack of change in FA detected in the present study could be a result of low level of general environmental stress on the contemporary population of Scandinavian wolves.

It is assumed that a large number of studies have failed to find a positive correlation between the level of genetic stress and developmental instability and have therefore never been published (Pertoldi et al. 2006). The current study is important in this context. The gray wolf population in Scandinavia is characterized by an extreme bottleneck followed by two and a half decades of strong inbreeding, but no associated change in FA was detected.

Table 7 Correlation in fluctuating asymmetry with year of birth in six bilateral traits measured on the skulls of Scandinavian gray wolves

Numbers in parenthesis are sample sizes used for estimating measurement errors (ME)

Trait	N	r	p
CBL	85	−0.091	0.409
OPF	82	−0.027	0.808
PL	96	0.029	0.780
P1-M2	99	0.001	0.992
I1-M2	98	0.035	0.734
ML	90	−0.056	0.599

Acknowledgments We are very thankful to Nina J.V. Juliussen who made all the measurements, to Anne-Karin Hufthammer for access to wolf skulls at the Bergen Museum, and to Ulf Johansson, Olavi Grönwall and Peter Mortensen for access to wolf skulls at the Naturhistoriska riksmuseet, Stockholm. Comments from two anonymous referees improved the manuscript substantially.

References

- Bechshøft TØ, Wiig Ø, Sonne C, Rigét FF, Dietz R, Letcher RJ, Muir DCG (2008a) Temporal and spatial variation in metric asymmetry in skulls of polar bears (*Ursus maritimus*) from East Greenland and Barents Sea. *Ann Zool Fenn* 45:15–31
- Bechshøft TØ, Rigét FF, Wiig Ø, Sonne C (2008b) Fluctuating asymmetry in metric traits: a practical example of calculating asymmetry, measurement error, and repeatability. *Ann Zool Fenn* 45:32–38
- Bensch S, Andrén H, Hansson B, Pedersen HC, Sand H, Sejberg D, Wabakken P, Åkeson M, Liberg O (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. *PLoS ONE* 1:e72
- Clarke GM (1998) The genetic basis of developmental stability. V. Inter- and intra-individual character variation. *Heredity* 80:553–561
- Flagstad Ø, Walker CW, Vilà C, Sundqvist A-K, Fernholm B, Hufthammer AK, Wiig Ø, Koyola I, Ellegren H (2003) Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. *Mol Ecol* 12: 869–880
- Gibson PS, Ballard WB, Nowak RM, Mech LD (2000) Accuracy and precision of estimating age of gray wolves by tooth wear. *J Wildl Manag* 64:752–758
- Hagenblad J, Olsson M, Parker HG, Ostrander EA, Ellegren H (2009) Population genomics of the inbred Scandinavian wolf. *Mol Ecol* 18: 1341–1351
- Hoelzel AR, Fleischer RC, Campagna C, Le Boeuf BJ, Alvord G (2002) Impact of a population bottleneck on symmetry and genetic diversity in the northern elephant seal. *J Evol Biol* 15:567–575
- Lacy RC, Alaks G (2013) Effects on inbreeding on skeletal size and fluctuating asymmetry of *Peromyscus polionotus* mice. *Zoo Biol* 32: 125–133
- Landon DB, Waite CA, Peterson RO, Mech LD (1998) Evaluation of age determination techniques for gray wolves. *J Wildl Manag* 62:674–682
- Leamy LJ, Meagher S, Taylor S, Carrol P, Potts WK (2001) Size and fluctuating asymmetry of morphometric characters in mice: their associations with inbreeding and t-haplotype. *Evolution* 55:2333–2341
- Leung B, Forbes MR (1997) Modelling fluctuating asymmetry in relation to stress and fitness. *Oikos* 78:397–405
- Liberg O, Andrén H, Pedersen H-C, Sand H, Sejberg D, Wabakken P, Åkesson M, Bensch S (2005) Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biol Lett* 1:17–20
- Lovatt FM, Hoelzel AR (2011) The impact of population bottlenecks on fluctuating asymmetry and morphological variance in two separate populations of reindeer on the island of South Georgia. *Biol J Linn Soc* 102:798–811
- Mattisson J, Sand H, Wabakken P, Gervasi V, Liberg O, Linnell JDC, Rauset GR, Pedersen HC (2013) Home range size variation in a recovering wolf population: evaluating the effect of environmental, demographic, and social factors. *Oecologia* 173:813–825
- Nowak RM (2003) Wolf evolution and taxonomy. In: Mech LD, Boitani L (eds) *Wolves. Behaviour, ecology, and conservation*. The University of Chicago Press, Chicago and London, pp 239–259
- Palmer AR (1994) Fluctuating asymmetry analyses: a primer. In: Markow TA (ed) *Developmental instability: its origins and evolutionary implications*. Kluwer, Dordrecht, pp 335–364
- Palmer AR, Strobeck C (1986) Fluctuating asymmetry—measurement, analysis, patterns. *Ann Rev Ecol Syst* 17:391–421
- Palmer AR, Strobeck C (2003a) Fluctuating asymmetry analyses revisited. In: Polak M (ed) *Developmental instability. Causes and consequences*. Oxford University Press, Oxford, pp 279–319
- Palmer AR, Strobeck C (2003b) Fluctuating asymmetry analysis: a step-by-step example. From: electronic Appendix V in: fluctuating asymmetry analyses revisited. In: Polak M (ed) *Developmental instability. Causes and consequences*. Oxford University Press, Oxford, pp 279–319
- Pertoldi C, Loeschcke V, Madsen AB, Randi E (1997) Developmental stability in the Eurasian otter (*Lutra lutra*) in Denmark. *Ann Zool Fenn* 34:187–196
- Pertoldi C, Loeschcke V, Braun A, Madsen AB, Randi E (2000) Craniometrical variability and developmental stability. Two useful tools for assessing the population viability of Eurasian otter (*Lutra lutra*) populations in Europe. *Biol J Linn Soc* 70:309–323
- Pertoldi C, Kristensen TN, Andersen DH, Loeschcke V (2006) Developmental instability as an estimator of genetic stress. *Heredity* 96:122–127
- Peters G (1993) *Canis lupus* Linnaeus, 1758 – Wolf. In: *Hanbuch der Säugetiere Europas. Band 5: Raubsäuger – Carnivora (Fissipedia) Teil I: Canidae, Ursidae, Procyonidae, Mustelidae* 1. AULA-Verlag, Wiesbaden, pp 47–106
- Pulliainen E (1985) The expansion mechanism of the wolf (*Canis lupus*) in Northern Europe. *Rev Ecol* 40:157–162
- Räikkönen J, Bignert A, Mortensen P, Fernholm B (2006) Congenital defects in a highly inbred wild wolf population (*Canis lupus*). *Mamm Biol* 71:65–73
- Vilà C, Sundqvist A-K, Flagstad Ø, Seddon J, Björnfeldt S, Kojola I, Casulli A, Sand H, Wabakken P, Ellegren H (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proc R Soc Lond B* 270:91–97
- Vøllestad LA, Hindar K, Mølle AP (1999) A meta-analysis of fluctuating asymmetry in relation to heterozygosity. *Heredity* 83: 206–218
- von den Driesch, A. 1976. A guide to the measurement of animal bones from archaeological sites. Harvard University, Peabody Museum of Archaeology and Ethnology, Peabody Museum Bulletin 1, pp148
- Wabakken P, Sand H, Liberg O, Björnvall A (2001) The recovery, distribution and population dynamics of wolves on the Scandinavian peninsula, 1978–1998. *Can J Zool* 79:710–725
- Wabakken P, Aronson Å, Strømseth TH, Sand H, Maartmann E, Svensson L, Flagstad Ø, Hedmark E, Liberg O and Kojola I (2011) The wolf in Scandinavia: Status report of the 2009–2010 winter. Høgskolen i Hedmark Oppdragsrapport nr. 4–2010, 57 pp
- Wabakken P, Svensson L, Kojola I, Maartmann E, Strømseth TH, Flagstad Ø, Åkesson, M and Zetterberg A (2013) The wolf in Scandinavia: Final report from wolf monitoring in the 2012–2013 winter. Høgskolen i Hedmark Oppdragsrapport nr. 5–2013, 34 pp