

CRANIAL MORPHOLOGY DYNAMICS IN A FOUNDER
POPULATION OF NORTH AMERICAN WOLVES

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Abstract

In this morphometric study, skulls of 34 adult gray wolves (*Canis lupus lycaon*) skulls from Isle Royale National Park (ISRO) and southern Ontario (ONT) were characterized by measurements of 54 skull traits. The Isle Royale wolf population was founded from the Ontario wolf population in the late 1940s, has remained reproductively isolated ever since, and is known to have lost significant genetic diversity. Using multivariate morphological data, we tested the following hypotheses regarding the evolutionary dynamics of founder effects in wild populations: 1) Mean size of ISRO wolves will be larger than ONT wolves because of consistently larger prey size; 2) the ISRO wolf population will possess less morphological variance in accordance with the hypothesis that genetic disparity is related to phenotypic disparity; 3) ISRO population, suffering from loss of genetic diversity, will possess greater fluctuating asymmetry (FA) due to loss of developmental stability.

There is little statistical evidence suggesting that ISRO wolves were larger. ISRO wolves possessed much greater morphological variance in some traits, conforming with some theoretical models and experiments, but generally contrary to conventional understanding of population genetics. ISRO wolves did not exhibit greater FA, and we question the use of FA as an index of developmental instability arising from loss of genetic variability.

Introduction

A founding population of gray wolves was established in Isle Royale National Park in the 1940s, an island (544 km² in area) in Lake Superior about 30 km from the Ontario (ONT) mainland. Genetic evidence indicates that the present population, effectively isolated had a single maternal ancestor, is heavily inbred, and has lost heterozygosity (Wayne et al. 1991).

Based on theoretical population genetic models we made several hypothesis regarding the morphological response of the population to the founder event and the resulting loss of genetic variability due to a founder event (Soule 1980). First, ISRO wolves should have a larger mean size than the ONT population in response to selective pressures associated with larger mean prey size (Macdonald 1983; Rosenzweig 1966). Large ungulate prey, which predominate in the wolf diet, include both white-tailed deer (*Odocoileus virginianus*) and moose in Ontario, but is limited to moose at ISRO. Schmitz & Kolenosky (1984) interpreted morphological differences between populations of wolves in Ontario partially as the result of differences in available prey size for wolves of southern Ontario.

Secondly, based on several theoretical models, we hypothesize that ISRO wolves will possess less morphological variation than ONT wolves. Only a subset of the alleles present in the parent population will be present in the founder population, and this loss of genetic variation (Mayr 1954, 1963, 1982), a loss of phenotypic variance (Soule 1980). It has been

estimated that the ISRO population has lost approximately 39-65% of the genetic heterozygosity present in the parent population (Wayne et al. 1991) leading to the expectation that morphological variance will likely be reduced. Some theoretical and experimental work with *Drosophila* has yielded results where population bottle necks have resulted in increased epistatic variance (Bryant et al. 1986 and Goodnight 1987) despite a loss of genetic heterozygosity. Our study design allows us to compare differences in morphological variability between two wild populations with known relative differences in genetic variability.

Thirdly, we hypothesize that ISRO wolves will exhibit a greater degree of fluctuating asymmetry (FA). FA is a specific type of asymmetry (differences between linear left and right measurements of bilaterally symmetrical traits) which arise when symmetry remains the normal state of the population, but individuals within the population exhibit asymmetry. FA is thought to arise from developmental instability as a consequence of genetic depauperacy and/or environmental stress, and thus it is considered to be useful to conservation biologists in detecting losses in population fitness (Leary and Allendorf 1989). One study found differences in cranial FA between cheetahs (*Acinonyx jubatus*) and four species of Felidae (Wayne et al 1986), and interpreted the results as supportive evidence of developmental instability due to genetic invariability. While another study found no differences in dental FA between cheetahs

and two different species of Felidae; interpreting these results as evidence not supportive of the hypothesis that developmental instability due to inbreeding exists in cheetahs (Keiser and Groeneveld 1991). However, for most species little is known of the relationship between FA and loss of genetic variability as well as which parts of vertebrate morphology are most sensitive to FA. We sought to establish the degree of FA in a genetically depauperate wolf population and to compare prevalence of FA in teeth vs. cranial traits.

Methods

We measured 54 variables on the skulls of 34 adult wolves from Isle Royale National Park and adjacent mainland Ontario (Table 1). Adult wolves were identified based on the closure of two sutures near the base of the skull (Peterson, pers. comm.). Measurements were made with calipers with a precision of 0.05mm. A random sample of 5% of all measurements taken were repeated to estimate measurement error. The absolute mean measurement error was 1.3%, and the errors were normally distributed. So we concluded that measurement error had little or no impact on the statistical results.

Multivariate methods were employed to detect morphological differences between the ISRO and ONT populations. Principle component analysis was used to select subsets of variables on which to run further multivariate analysis. Given the multivariate test results, univariate tests regarding differences

in mean size, variance, and FA were employed on all 54 measurements to aid interpretation.

Three subsets of variables were used in the multivariate analysis, corresponding to measurements taken of the skull, jaw, and teeth. Principle component analysis validated the selection of skull, jaw, and teeth subsets, as the cumulative percent variation represented by the first three principle components for each subset was high (Table 2).

Multivariate differences in mean skull size were evaluated using a MANOVA where sex and location (ISRO vs. ONT) were selection criteria. Univariate t-tests of location differences were employed to aid in interpreting the nature of the differences.

Detection of multivariate differences in variance were conducted by testing for equality of variance-covariance matrices. Univariate tests for difference in variance were also conducted to aid in interpretation.

Fluctuating asymmetry was measured by calculating left minus right differences divided by the mean measure of that trait (Leary and Allendorf 1989). We then tested whether this mean was equal to zero, as a test of directional asymmetry. We tested for anti-symmetry by evaluating normality of the distribution of left minus right difference for each individual within each variable. Corrections were introduced to cancel directional asymmetry and anti-symmetry, if needed (this was required only for two traits which were not significant in any differences between the

populations), then we evaluated population differences with a test for differences in the variance of left-minus-right measures among the individuals for each trait (Owen and McBee 1990).

These tests for differences in FA were carried out according to multivariate and univariate procedures.

Results

MANOVA results for differences in size when sex and location (ISRO vs. ONT) were included in the model indicated no differences in multivariate mean size in the Skull principle component (PC) or the Jaw PC. Only in the Teeth PC were evident differences in multivariate means, for both sex and location (Table 2). Univariate tests according to location show that only 8 of 54 traits were significantly different (Table 3). These results do not strongly contradict a null hypothesis of no differences in mean with an $\alpha=0.05$ (from an experiment-wise error rate of 0.05). Five of the eight differing traits were of teeth, from a table of 11 teeth traits measured. In half the cases IR population has a larger mean. In all but two cases the percent difference in means was less than 5.0%.

A multivariate test of equality of variance-covariance matrices was used to test to hypothesis that the morphological variance between ISRO and ONT wolves are equal (Barker 1984). The test yielded p-values of 0.004, 0.003, 0.044 for the skull, jaw, and teeth data subsets, for a hypothesis of equality of variance-covariance matrices. Univariate tests for differences

in individual traits show that only 8 of 54 traits have statistically significant differences in variance (Table 4). In all cases the IR population has the greater variance and the percent difference in variation always exceeds 40%.

The degree of FA in a trait is measured by the amount of variance in the distribution of left-minus-right measures of bilaterally symmetrical traits, therefore a comparison of variance-covariance matrices of these distributions is appropriate to test for multivariate differences in FA. The test yielded a p-value of <0.001 , for a hypothesis of equality of variance-covariance matrices. Univariate tests of individual traits indicate that only Tl1t (& Tlrt) and Acrt (& Ac1t) show statistically significant differences in FA among the populations. The degree of FA in the ISRO population was 2.0 and 4.0 times greater than the ONT population for each of these traits respectively.

Discussion

There is little or no evidence that IR wolves have diverged significantly in size in the four decades (4-6 generations) of isolation from the ONT parent population. Either selection pressures or time, or both have been insufficient for an increase in wolf body size that might result from increased prey size. Alternatively, or additionally, perhaps a founder effect and random genetic drift promoting the inheritance of smaller wolves were more powerful countervailing pressures.

Although only a small proportion of traits differed in the amount of morphological variance between ISRO and ONT wolves, these differences were large. The increased variance of the ISRO wolves across all traits which differed is contrary to our predictions. According to one model of population bottlenecks (Goodnight 1986) these results could be explained as follows: A founder event followed quickly by a population flush may result in the loss of a large proportion of the alleles present in the parent population, although the amount of heterozygosity need not be significantly less. In the parent population the source of most of the phenotypic variation will be a small number of common alleles. The distribution of alleles will be quite different in the founder population. Through processes of recombination and random founder events combinations of alleles with extreme phenotypic values may become present in greater proportion in the founder population than in the parent population, leading to, greater phenotypic variance in the former.

The possibility of these processes occurring as suggested by our data is not supportive of the hypothesis that phenotypic variability is proportionally related to genetic variability as stated by some models (references), nor is it supportive of hypotheses stating that developmental stability is proportionally related to genetic variability (references).

Introgression of coyote (*Canis latrans*) genes may have contributed to observed differences in morphological variability

between IR and ONT wolves. All ISRO wolves sampled have a single mtDNA genotype that is very rare on the ONT mainland, where several wolf and coyote mtDNA genotypes are represented (Wayne et al 1991). Possibly different levels of coyote nuclear gene introgression in ISRO and ONT wolves exists, due to founder effects.

No observable differences in FA between the two populations suggests either 1) developmental stability has not been lost despite a significant loss of genetic variability, or 2) high levels of canalization in wolves are present, capable of buffering against developmental instability despite loss of genetic variability.

There is little or no evidence that the ISRO wolves exhibit greater FA, in spite of an approximately 50% loss of genetic heterozygosity. This degree of genetic depauperacy may be contributing to loss of population fitness (Reference:Chap.---), that endangers the IR wolf population. We urge caution in the use of FA as the sole index of genetic stress or population fitness.

These results imply that measures of FA or morphologic variability may not be useful measures of genetic stress or developmental instability without some knowledge of thresholds in developmental biology and genetic condition of a species. It may be that species may possess inherent differences in FA and morphological variability unrelated to genetic stresses that are of interest to conservation biologists. Perhaps morphological

characters differ in sensitivity to FA, to be expected if differential selection pressure results in variation in degree of canalization.

Our results underscore new uncertainties in inferences regarding the population genetic histories based on morphology of the cheetahs based on the data they gathered, as in the case of cheetahs (Wayne et al 1986, Kieser and Groenvelde 1991). More populations with known genetic histories should be studied to test hypotheses regarding the expression of FA and developmental instability. In the future, development of models based on properties of drift, selection, and heritability could provide insight to the underlying processes of the evolutionary dynamics of IR wolves with regard to their morphologic size and variation. The ISRO wolf population merits continued study in order to track FA and morphological variability in the face of continued loss of genetic variability.

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Table 1. 54 Skull traits measured.

<u>Trait</u>	<u>Description</u>
SLs*	skull length to the sagital crest
SH*	skull height
SWz*	skull width at zygomatic arch
SWp*	post orbital process maximum width
SWc*	skull width at point of canine canine
SWt*	skull width at temporal fossa
OCw2	width of both occipital condyls
SLo	skull length to occipital condyl
SWx	skull width at protocone of upper carnisial
M1LW	width of M ₁
P4LW	width of P ₄
P4UL***	length of P ₄
P4UW***	width of P ₄
JHr**	ramus height between insertion point of P ₄ and M ₁
JLc**	Jaw length to the condyl
RW**	ramus width of jaw at metacone of M ₁

Bilaterally Symmetrical Measurements

CART & CALt	centerline of skull TO point of canine insertion in skull (buccal side).
P4Art & P4Art	centerline of skull TO point of P ₄ insertion in skull (buccal side).
TLrt*** & TLlt	length of upper tooth row (canine to M ²)
STrt & STlt	jaw condyl receptor in skull TO inside anterior edge of zygomatic arch
PZrt & PZlt	posterior of palatine TO inside posterior edge of zygomatic arch
PCrt & PClt	paroccipital process TO posterior edge of condyl receptor
PPrt & PPlt	centerline of skull TO paroccipital process
VZrt & VZlt	vertebral foramen TO inside posterior edge of zygomatic arch
JLart** & JLalt	jaw length to angular process
JHart** & JHalt	angular process TO top of jaw

CWlrt & CWllt	width of jaw condyl along long axis
ASrt & ASlt	Audial foramen TO top of sagital crest
MZrt & MZlt	base of tooth between M^1 and M^2 TO highest point on zygomatic arch
M1ULrt & M1ULlt***	length of M^1
M1LLrt & M1LLlt	length of M_1
P4LLrt & P4LLlt	length of P_4
OCwlrt & OCwllt	width of occiptal condyl
OClrt & OCllt	length of occiptal condyl
CWsrt & CWSlt	width of jaw condyl along short axis
ACrt & AClt	Angular process TO condyl of jaw

*Traits included on the skull subset of data (see text).

**Traits included on the jaw subset of data (see text).

***Traits included on the teeth subset of data (see text).

Table 2. MANOVA test results for hypotheses of population difference in multivariate mean.

Principle Component Name	Traits in PC	Cumulative Variation of First 3 PC's	p-value for sex	p-value for location
Skull	Sls, Sh, Swz, Swp, Swc, Swt	0.92	0.383	0.619
Jaw	Jlc, Jhr, Rw, Jlar, Jhart	0.97	0.1327	0.6796
Teeth	Tlrl, P4uw, P4ul, M1ullt	0.94	.0350	.0012

Table 3. Univariate test results for difference in mean size.

Trait	Population with greater mean*	Percent difference in mean
M1ul	Ont	4.7
Mlw	Ont	3.7
P4lw	Ont	1.9
Rw	Ont	7.8
P4uw	IR	13.1
P4ll	IR	3.3
Vz	IR	2.5
Ocw	IR	4.1

*alpha=0.10

Table 4. Univariate test results for population differences in variance.

Trait	Population with greater variance*	Percent difference
Sh	IR	45.0
Swt	IR	49.1
Jhr	IR	54.8
Strt	IR	44.0
Stlt	IR	60.0
Pp	IR	41.0
Mzlt	IR	56.0
Mzrt	IR	74.0

*alpha=0.10