Morphological differences between different morphs of Arctic charr *(Salvelinus alpinus)*

Hlynur Á. Þorleifsson

Líf- og umhverfisvísindadeild
Háskóli Íslands
2015

Morphological differences between different morphs of Arctic charr *(Salvelinus alpinus)*

Hlynur Á. Þorleifsson

10 eininga ritgerð sem er hluti af
Baccalaureus Scientiarum gráðu í líffræði

Leiðbeinandi
Zophonías Oddur Jónsson
Kalina Hristova Kapralova

Líf- og umhverfisvísindadeild
Verkfræði- og náttúruvísindasvið
Háskóli Íslands
Reykjavík, Maí 2015

Morphological differences between different morphs of Arctic char *(Salvelinus alpinus)*
Breytileiki á útliti mismunandi afbrigða af bleikju í Þingvallavatni.

10 eininga ritgerð sem er hluti af *Baccalaureus Scientiarum* gráðu í líffræði

Líf- og umhverfisvísindadeild
Verkfræði- og náttúruvísindasvið
Háskóli Íslands
Askja, Sturlugata 7
107 Reykjavík
Sími: 525 4000

Skráningarupplýsingar:
Hlynur Á. Þorleifsson, 2015, *Morphological differences between different morphs of Arctic char (Salvelinus alpinus)*, BS ritgerð, Líf- og Umhverfisvísindadeild, Háskóli Íslands, 30 bls.

Prentun: Háskólaprent
Reykjavík, Maí 2015

Útdráttur

Það finnast fjögur afbrigði af bleiku í Þingvallavatni í dag. Þær er skilgreindar eftir útlitseinkennum, svo sem stærð og mun á lögun höfuðs. Þessi fjögur afbrigði eru: Dvergbleikja, kuðungableikja, sílableikja og murta. Í þessari rannsókn, var breytileiki á milli afbrigða og blendinga þeirra athugaður í 1 árs gömlum seiðum sem alin höfðu verið við sömu aðstæður, með geometrískum, formfræðilegum aðferðum.

Breytileiki á milli afbrigða og blendinganna er til staðar og virðist vera að það séu möguleg bæði móður og föður áhrif sem hafa áhrif á útlitseinkenni afkvæmis. Það er marktækur munur á milli sumra afbrigðana og kynblendinga.

Abstract

There are four morphs of arctic charr *(Salvelinus alpinus)* present in Þingvallavatn. They can be classified according to phenotype, such as size, and ecotypical differences. The four morphs are: small benthivorous (SB), large benthivorous (LB), planktivorous (PL) and piscivorous (PI). In this study, differences between morphs and their hybrids were investigated for 1-year-old junveniles, reared in a “common garden” environment, using geometric morphometrics techniques.

The difference between the morphs and their hybrids is present and it seems that both maternal and paternal influences affect their offspring. There is significant difference between some morphs and hybrids.

Efnisyfirlit

Þakkir

Ég vil þakka Zophoníasi O. Jónssyni og Kalinu Hristovu Kapralovu fyrir alla hjálpina við útreikninga og frágang á þessu verkefni og einnig þakka Berglindi Dögg Ómarsdóttur fyrir hjálp við gagnaúrvinnslu.

1 Introduction

In Þingvallavatn in southern Iceland there are four coexisting morphs of Arctic charr *(Salvelinus alpinus),* each one very different from the others both in physical attributes and life-history characteristics, such as feeding, growth and age at sexual maturity. The four morphs are large benthivorous (LB), small benthivorous (SB), piscivorous (PI) and planktivorous (PL) **(**Skúlason et al., 1989**)**. These differences in morphology clearly relate to feeding habits and habitat selection *(see table 1)*. The Icelandic Artic charr originated from a single Atlantic lineage and this species shows a very high level of variation in phenotypes between populations and many examples of polymorphism have been documented **(**Kapralova, 2014**)**.

All the morphs spawn in the stony littoral habitat but the timing of spawning is different between morphs. Interbreeding among morphs does exist, and in the case of the smallest one (PL and SB), interbreeding opportunities seem possible **(**Kapralova, 2014**).**

In this study we investigate the physical differences between 1 year old juveniles from different morphs of Þinvallavatn Arctic charr and their hybrid crosses, juveniles using geometric morphometrics. We also investigated the differences in shape between pure and hybrid crosses from different populations of SB around Iceland. All juveniles were reared under identical conditions in "Verið" aquaculture facilities in Sauðárkrókur.

Thus the two questions asked in this study were:

Question 1: Are the morphs in Þingvallavatn and their hybrids different in shape?

Question 2: Are the SB crosses from various locations different in shape?

Table 1: Characteristics of the four morphs of *Salvelinus alpinus* in Þingvallavatn.

	Morph			
Character	LB	SB	PI	PL
Icelandic name	Kuðungableikja	Dvergbleikja	Sílableikja	Murta
Age of sexual maturity (years)	3-11	2-4	5-10	3-5
Size at maturity (cm)	20-50	7-15	25-60	15-22
Body morphology	Blunt snout, Short lower jaw, stocky body, long fins	Similar to LB	Pointed snout, equal jaw length Streamlined, short fins	Similar to PI, relatively shorter and compact jaw

2 Materials and method

For this study, 601 individual were photographed and 21 landmarks placed on predetermined areas *(see figure 1).* Fish were collected in fall 2012, in Þingvallavatn and various other locations is southern Iceland and pure and hybrid crosses were created. Juvenile fish were photographed in the fall of 2013. For the landmarking, TpsUtil was used to create a tps file for all the individuals and Tpsdig 2 was used to mark the landmarks of each spot picked *(see figure 1).* To assess the repeatability of finding and positioning of the landmarks (data quality), 20 random individuals were scored for the 21 landmarks two times. The difference between the landmark sessions was assessed using Discriminant Function Analysis (DFA). The difference between means was not significant (p=0,5210, 1000 permutations). All analyses were done in MorphoJ.

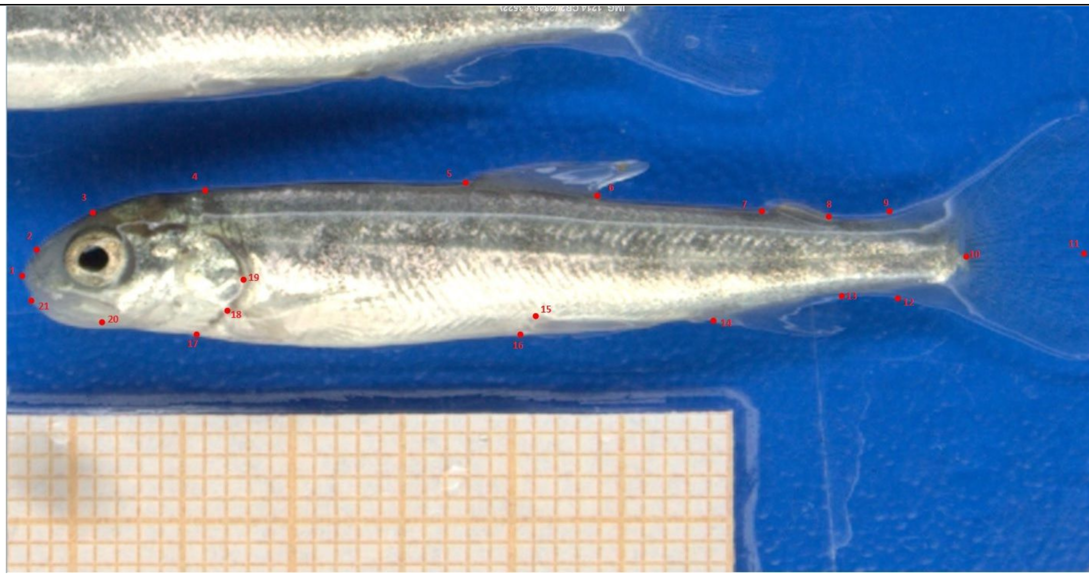

Figure 1: *Landmark placement. Numbered. Started with the front and worked around, ending with the mouth. Measurement scale can be seen, one box equals 1 mm. It's a cross, PlxPI. It's mother was a* planktivorous and the father is piscivorous.

Table 2: Number of individulas per each morph from Þinvallavatn.: LB (Large Benthic), SB (Small Benthic), PL (Planctivorous), PI (Piscivorous) , PixSB (hybrid cross between PI female and SB male), PLxSB (hybrid cross between PL female and SB male and PlxPI (hybrid cross between PL female and PI male).

Groups	Observations	
1	LB	55
2	PI	42
3	PIxSB	48
4	PL	177
5	PLxPI	38
6	PLxSB	86
7	SB	37

Table 3: List of hybrid and pure SB from different locations

SB	**HUxMI**	**HUxSR**	**L7xL7**	**MIxHU**	**MIxMI**	**MIxSD**	**MIxSR**	**MIxTH**	**SBxSB**	**HUxMI**	**THxKA**
Hybrid or Pure	Hybrid	Hybrid	Pure	Hybrid	Pure	Hybrid	Hybrid	Hybrid	Pure	Hybrid	Hybrid
Mother is from:	HU	HU	Not known	MI	MI	MI	MI	MI	SB	HU	TH
Father is from:	MI	SR	Not known	HU	MI	SD	SR	TH	SB	MI	KA

3 Results

3.1 Procustes fit

All studies in geometric morphometrics are based on configurations of landmarks. These analyses consider the arrangement of landmarks relative to one another. The Procrustes fit uses all landmarks to fit the configurations to each other optimally after all configurations have first been scaled to have a centroid size of 1.0. The criterion for the best fit is usually the minimal sum of squared distances between corresponding landmarks. This overall fit automatically aligns the configurations so that they have a standard position and orientation ("Shape of Landmark", n.d., para 1,3**).**

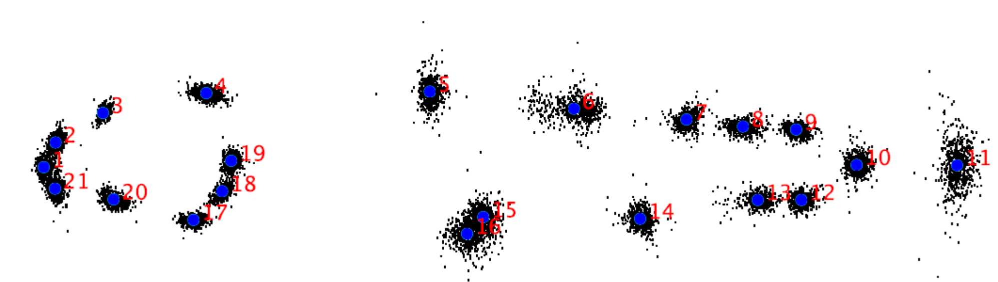

Figure 2: *Procrustes fit. It is apparent that points 6 and 11 are the ones with the most wide range placement. Point 11 can be explained with the bending of the tail. It is harder to explain why point 6 is so spread out.*

3.2 Principal Component Analysis (PCA)

PCA is a technique for evaluating the overall variation in a dataset. It can be used to see whether there are obvious subdivisions, but it may miss such subdivisions even if they are present. Importantly, PCA can also be used to see which shape changes are associated with the most variation or the least variation to identify shape features that are particularly variable or particularly constant.("Principal components", n.d. para 2)

3.2.1 PCA Morphs

Table 4: The first 12 components describe 90% of the variance with the first 3 describing 61% of the variance

	Eigenvalues	% Variance	Cumulative %
1.	0.00045221	35.451	35.451
2.	0.00018694	14.655	50.107
3.	0.00013647	10.699	60.805

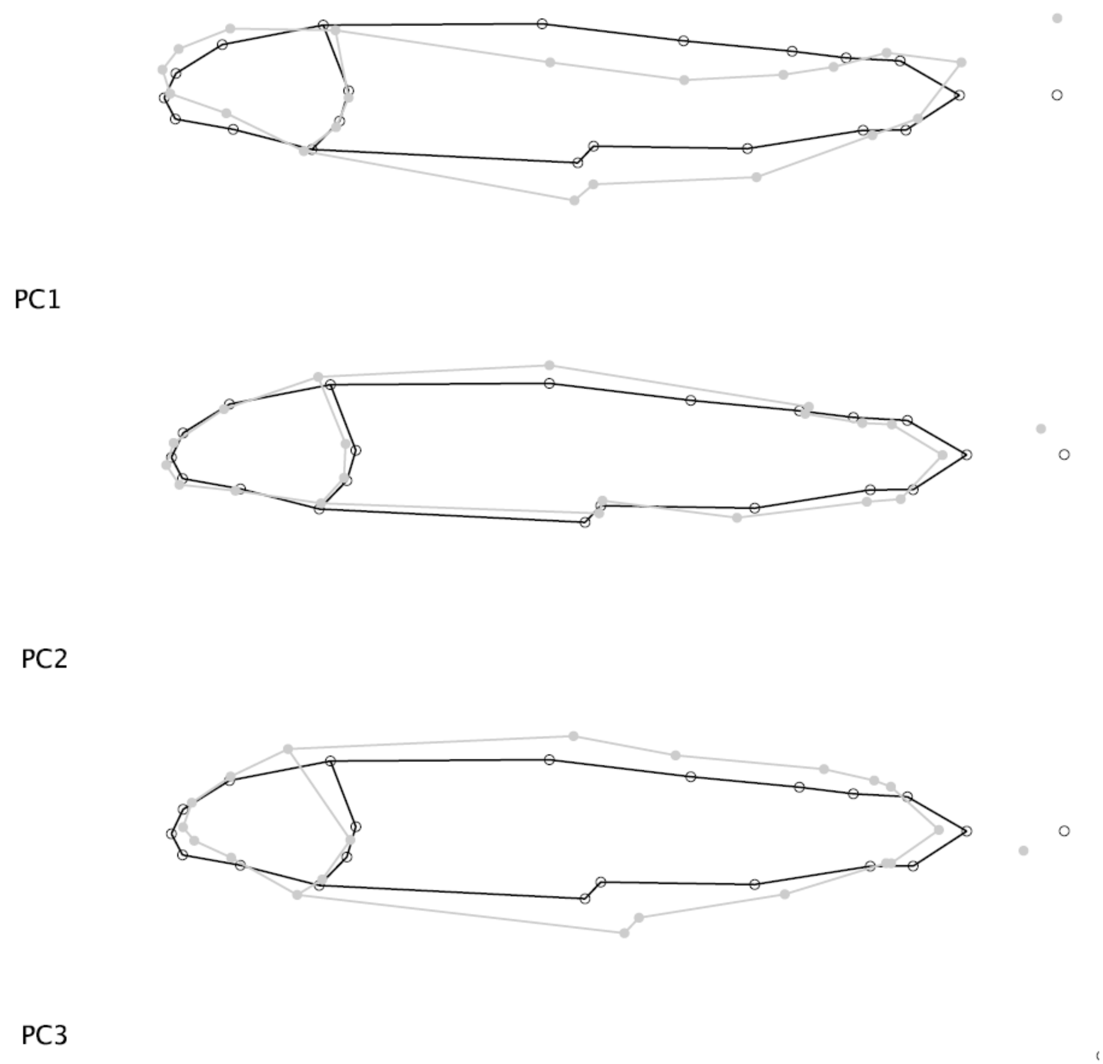

Figure 3: *PC1 catches the bending of the samples and explains 35% of the variance. This means that some of the samples are bent but (nor surprisingly) bending is not morph specific (see figure 4). Shape changes associated are shown with wireframes, black is the starting shape (i.e the -) and grey is the +*

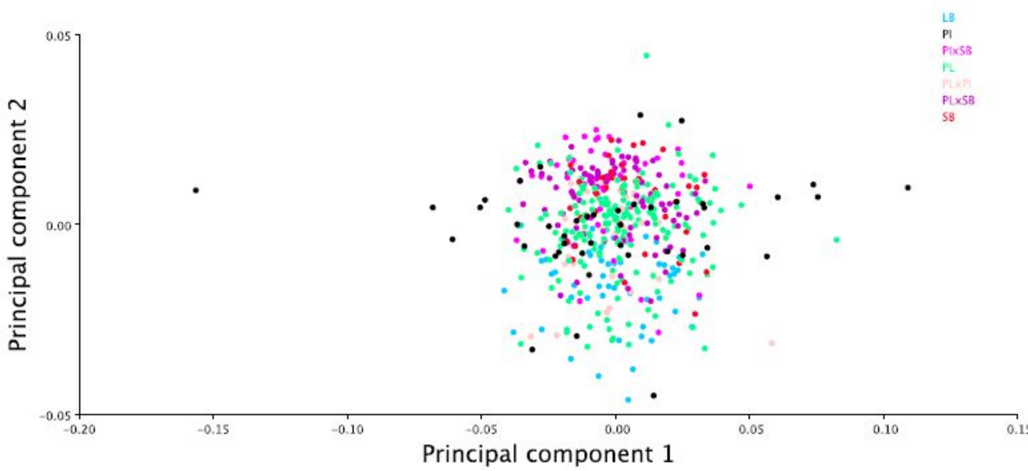

Figure 4: *shows that bending is not morph specific and no morph is bending more than another, except some extremes from PI, which were removed from further analyses.*

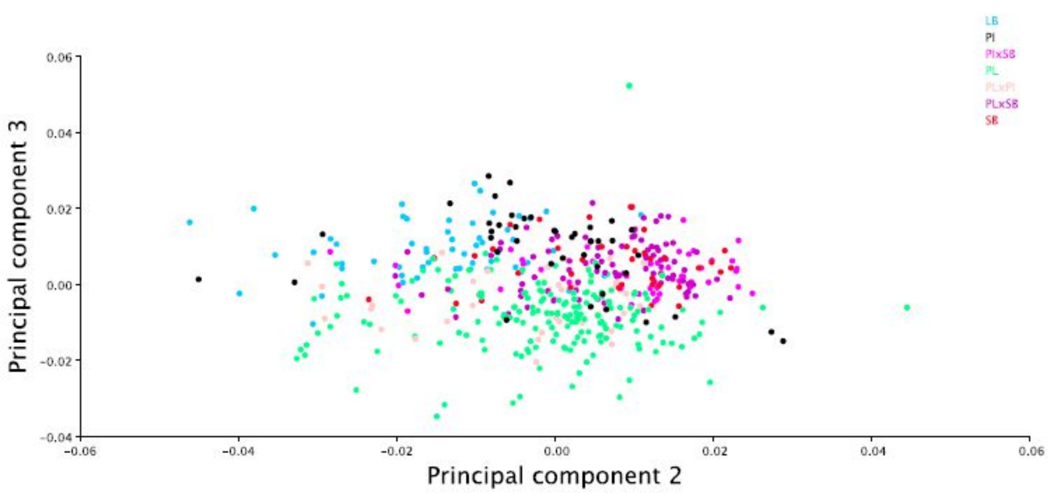

Figure 5: *PC2 seems to be separating PL and all the SB+ hybrids from LB. PC3 separates LB and PL.*

3.2.2 PCA on dwarfs from different ponds and dwarf crosses

Juveniles SB from various parts of Iceland (see table 3).

Table 5: The first 9 components describe 90% of the variance with the first 3 desribing 68% of the variance

	Eigenvalues	% Variance	Cumulative %
1.	0.00074117	43.441	43.441
2.	0.00023038	13.503	56.944
3.	0.00018597	10.900	67.844

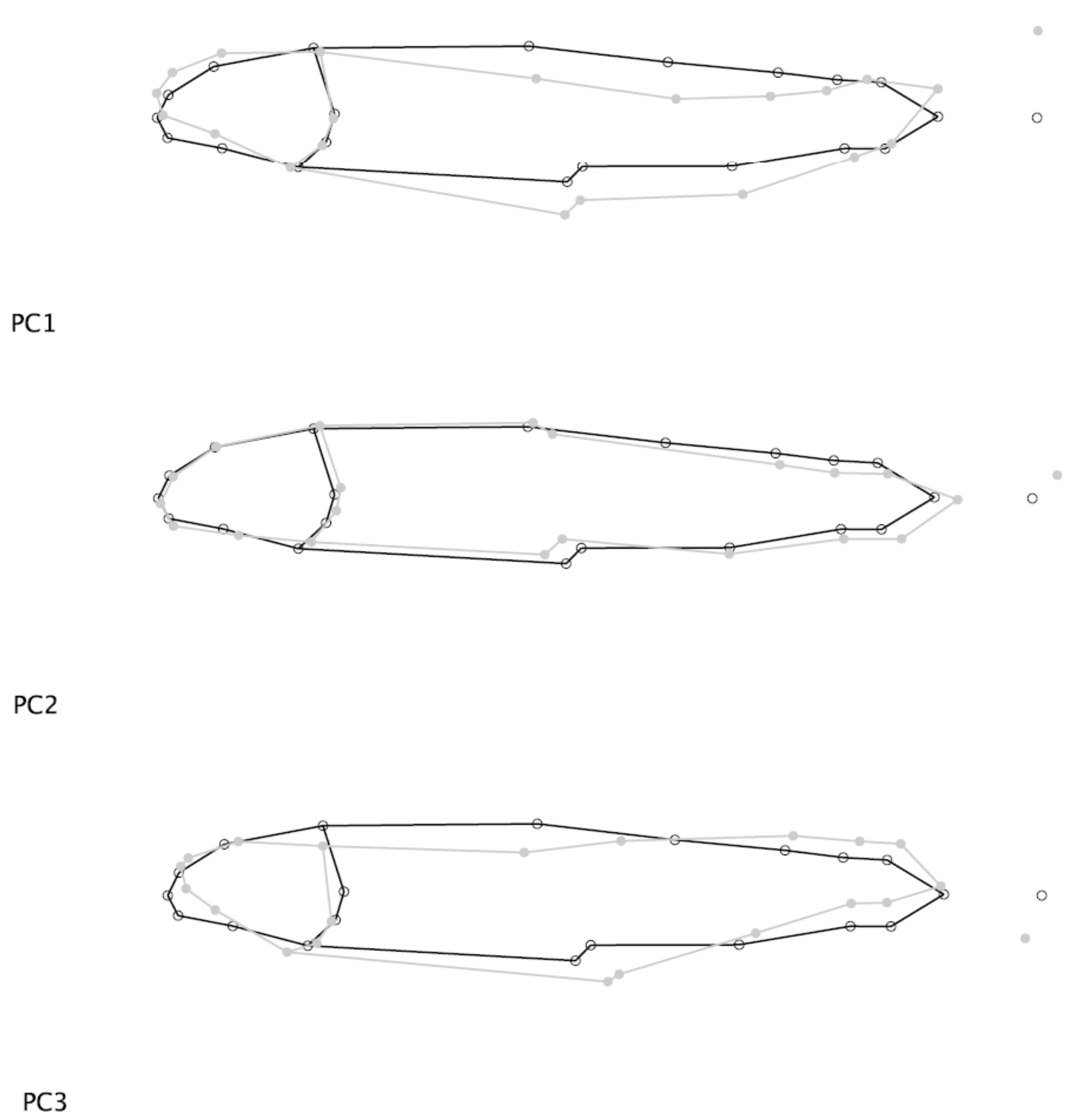

Figure 6: PC1 catches the bending of the samples and explains 43% of the variance. More of the cross samples are bent and there doesn't seem to be any cross that bends more than others (see figure 7). Bending is something that happened during photographing. No morph was bended more than another, at least not on purpose.

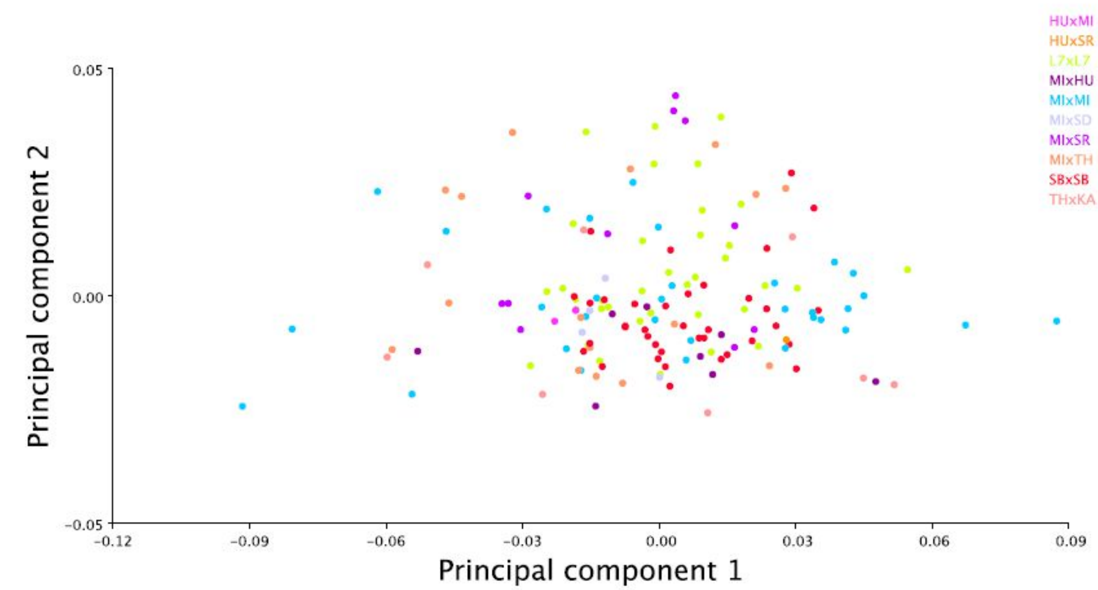

Figure 7: *No cross is more prone to bending than others.*

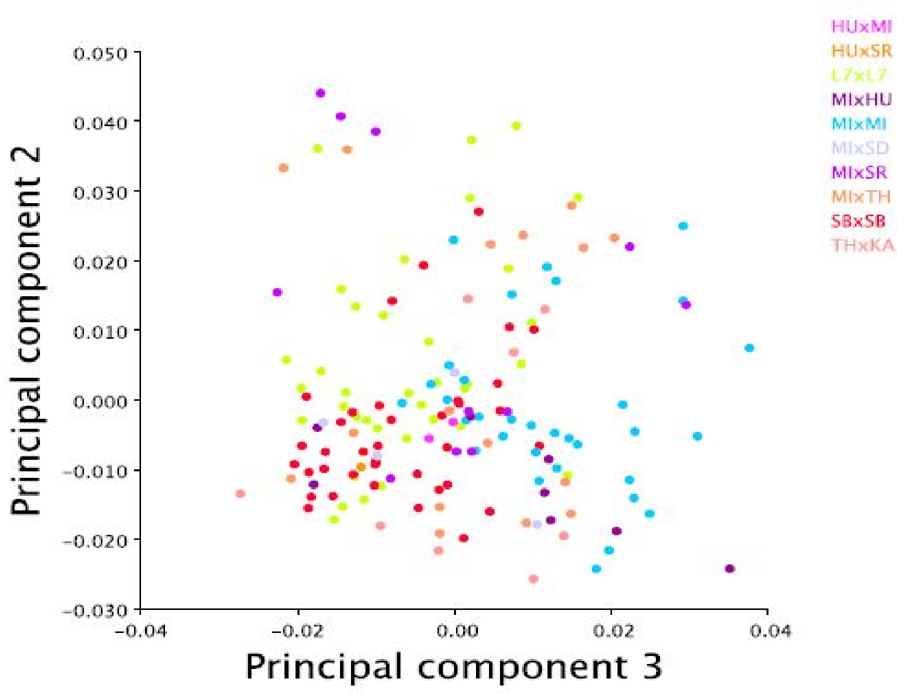

Figure 8: *PC3 appears to separate MIxMI from SBxSB*

3.3 Canonical variate analysis (CVA)

CVA is a technique to visualize differences among groups. The purpose is to find relationship between two points. It's done by finding the linear combination of those two points which are most highly correlated. Everything is scaled so that the variance equals 1. Mahalanobis distance measures the distance of separation between those two points. (Tofallis, 1999)

3.3.1 CVA Morphs

Table 6: Variation among groups, scaled by the inverse of the within-group variation

	Eigenvalues	% Variance	Cumulative %
1	5,20126487	54,039	54,039
2	1,81162057	18,822	72,862
3	1,59412488	16,562	89,424
4	0,49949545	5,19	94,614
5	0,40805252	4,24	98,853
6	0,11037767	1,147	100

Table 7: Mahalanobis distances among groups

	LB	PI	PIxSB	PL	PLxPI	PLxSB
PI	6,0364					
PIxSB	5,9658	5,3503				
PL	7,1956	5,9574	4,1925			
PLxPI	7,3407	5,7884	4,0749	2,9164		
PLxSB	5,7164	4,9724	2,0158	3,6908	3,7067	
SB	6,0992	5,4312	3,0808	4,2489	4,2775	1,8546

P-values from permutation tests (10000 permutation rounds) for Mahalanobis distances among morphs were all under 0.0001

Table 8: Procrustes distances among groups

	LB	PI	PIxSB	PL	PLxPI	PLxSB
PI	0,0252					
PIxSB	0,0278	0,0227				
PL	0,0282	0,0291	0,0202			
PLxPI	0,0243	0,0231	0,0179	0,0128		
PLxSB	0,0251	0,0188	0,0074	0,0198	0,0158	
SB	0,0256	0,0203	0,011	0,021	0,0188	0,0069

Table 9: P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups:

	LB	PI	PIxSB	PL	PLxPI	PLxSB
PI	<.0001					
PIxSB	<.0001	0,0006				
PL	<.0001	<.0001	<.0001			
PLxPI	<.0001	0,0017	<.0001	0,0005		
PLxSB	<.0001	0,0004	0,0526	<.0001	<.0001	
SB	<.0001	0,0092	0,0093	<.0001	<.0001	0,1271

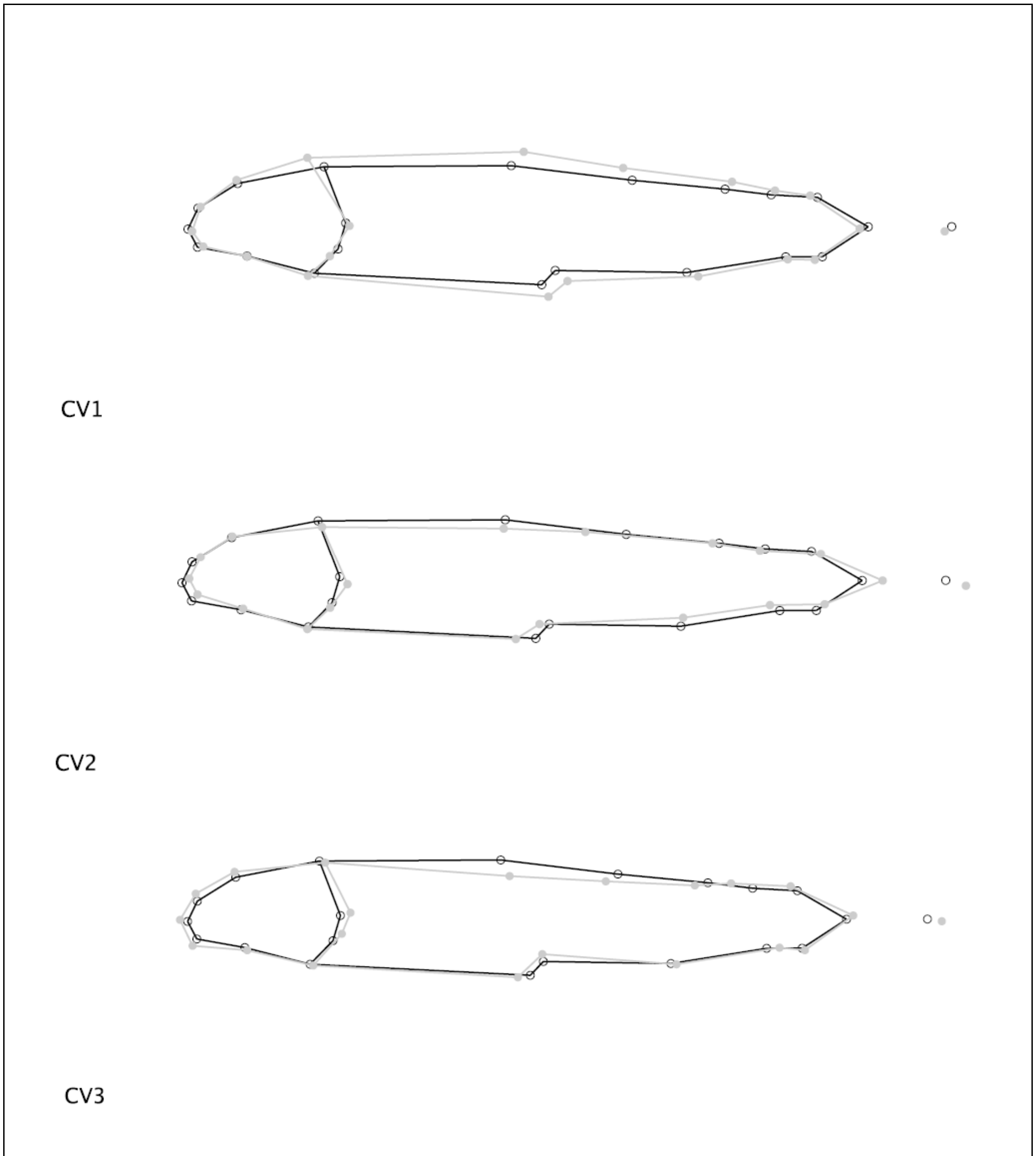

Figure 9: CV1 shows most variability in shape.

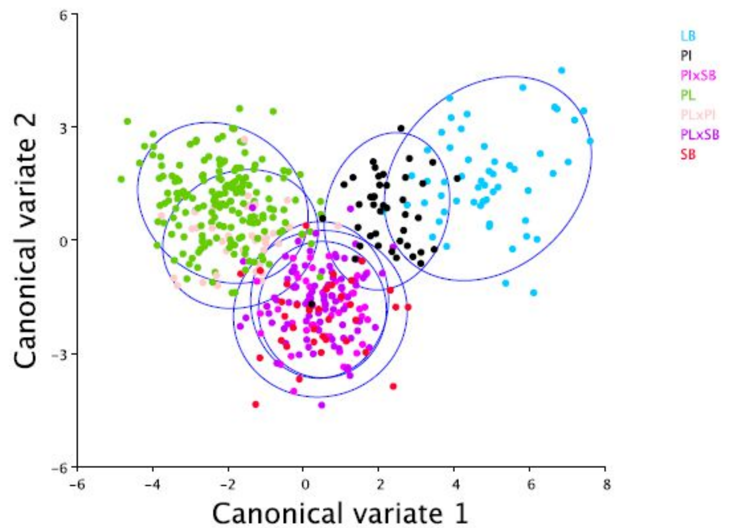

Figure 10: CV1 separates LB and PI from PL. Hybrids between PL and PI seem to follow their maternal phenotype. PLxSB on the other hand seems to follow their paternal phenotype, if compared to SB

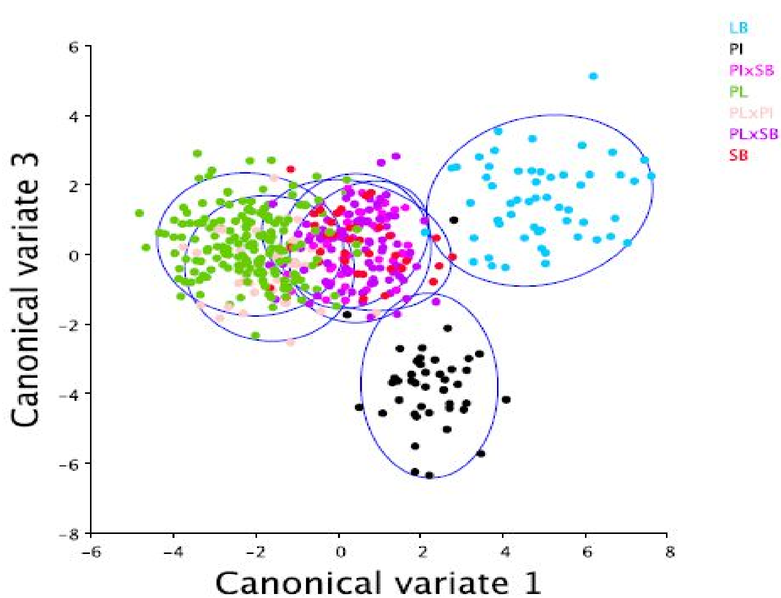

Figure 11: See similar separation here between CV1 and CV3.

3.3.2 CVA on dwarfs from different ponds and dwarf crosses

Table 8: Variation among groups, scaled by the inverse of the within-group variation

	Eigenvalues	% Variance	Cumulative %
1	7,23873884	40,749	40,749
2	4,68923013	26,397	67,145
3	2,24389851	12,631	79,777
4	1,32766308	7,474	87,25
5	1,00395284	5,651	92,902
6	0,48243203	2,716	95,618
7	0,42413961	2,388	98,005
8	0,22568752	1,27	99,276
9	0,12866413	0,724	100

Table 11: Mahalanobis distances among groups

	HUxMI	HUxSR	L7xL7	MIxHU	MIxMI	MIxSD	MIxSR	MIxTH	SBxSB
HUxSR	7,6257								
L7xL7	7,4003	9,1006							
MIxHU	7,0211	8,6359	6,9016						
MIxMI	5,1377	8,4671	6,6869	3,7708					
MIxSD	5,9766	8,8354	6,5444	6,5806	6,1416				
MIxSR	5,3083	6,5456	7,8815	5,49	4,7246	6,2073			
MIxTH	5,9036	8,0806	7,3815	5,4056	5,4242	5,2925	4,8267		
SBxSB	5,3748	7,3386	5,9653	6,2779	5,9373	5,712	5,7066	5,3573	
THxKA	8,2134	8,5607	9,1536	6,7529	8,0488	8,0106	6,7915	5,9138	6,6182

Table 12: P-values from permutation tests (10000 permutation rounds) for Mahalanobis distances among groups

	HUxMI	HUxSR	L7xL7	MIxHU	MIxMI	MIxSD	MIxSR	MIxTH	SBxSB
HUxSR	0,3379								
L7xL7	0,0001	0,032							
MIxHU	0,0018	0,0111	<.0001						
MIxMI	0,1031	0,0528	<.0001	<.0001					
MIxSD	0,219	0,05	<.0001	0,0002	<.0001				
MIxSR	0,0207	0,1024	<.0001	<.0001	<.0001	0,0007			
MIxTH	0,0223	0,022	<.0001	<.0001	<.0001	<.0001	<.0001		
SBxSB	0,0157	0,0503	<.0001	<.0001	<.0001	0,0001	<.0001	<.0001	
THxKA	0,0028	0,0449	<.0001	0,0001	<.0001	0,001	<.0001	<.0001	<.0001

Table 13: Procrustes distances among groups

	HUxMI	HUxSR	L7xL7	MIxHU	MIxMI	MIxSD	MIxSR	MIxTH	SBxSB
HUxSR	0,0551								
L7xL7	0,0294	0,043							
MIxHU	0,0333	0,0467	0,0314						
MIxMI	0,0315	0,0464	0,0261	0,0166					
MIxSD	0,0166	0,0468	0,0229	0,0267	0,0269				
MIxSR	0,03	0,049	0,0274	0,0314	0,0255	0,0263			
MIxTH	0,0224	0,0524	0,0271	0,0272	0,0254	0,0202	0,0176		
SBxSB	0,0326	0,0348	0,0209	0,0231	0,0249	0,0252	0,029	0,0275	
THxKA	0,0364	0,0471	0,0351	0,0189	0,0271	0,0314	0,0302	0,0283	0,0236

Table 14: P-values from permutation tests (10000 permutation rounds) for Procrustes distances among groups

	HUxMI	HUxSR	L7xL7	MIxHU	MIxMI	MIxSD	MIxSR	MIxTH	SBxSB
HUxSR	0,3379								
L7xL7	0,1659	0,1431							
MIxHU	0,264	0,3896	<.0001						
MIxMI	0,4089	0,3294	0,0008	0,448					
MIxSD	0,9134	0,1912	0,1089	0,22	0,3051				
MIxSR	0,2848	0,0065	<.0001	0,0173	0,077	0,1516			
MIxTH	0,7596	0,2339	<.0001	0,0588	0,0404	0,537	0,2754		
SBxSB	0,0237	0,1189	<.0001	0,0039	0,0001	0,0106	<.0001	<.0001	
THxKA	0,389	0,4945	0,0003	0,5072	0,1327	0,2654	0,0858	0,0916	0,0128

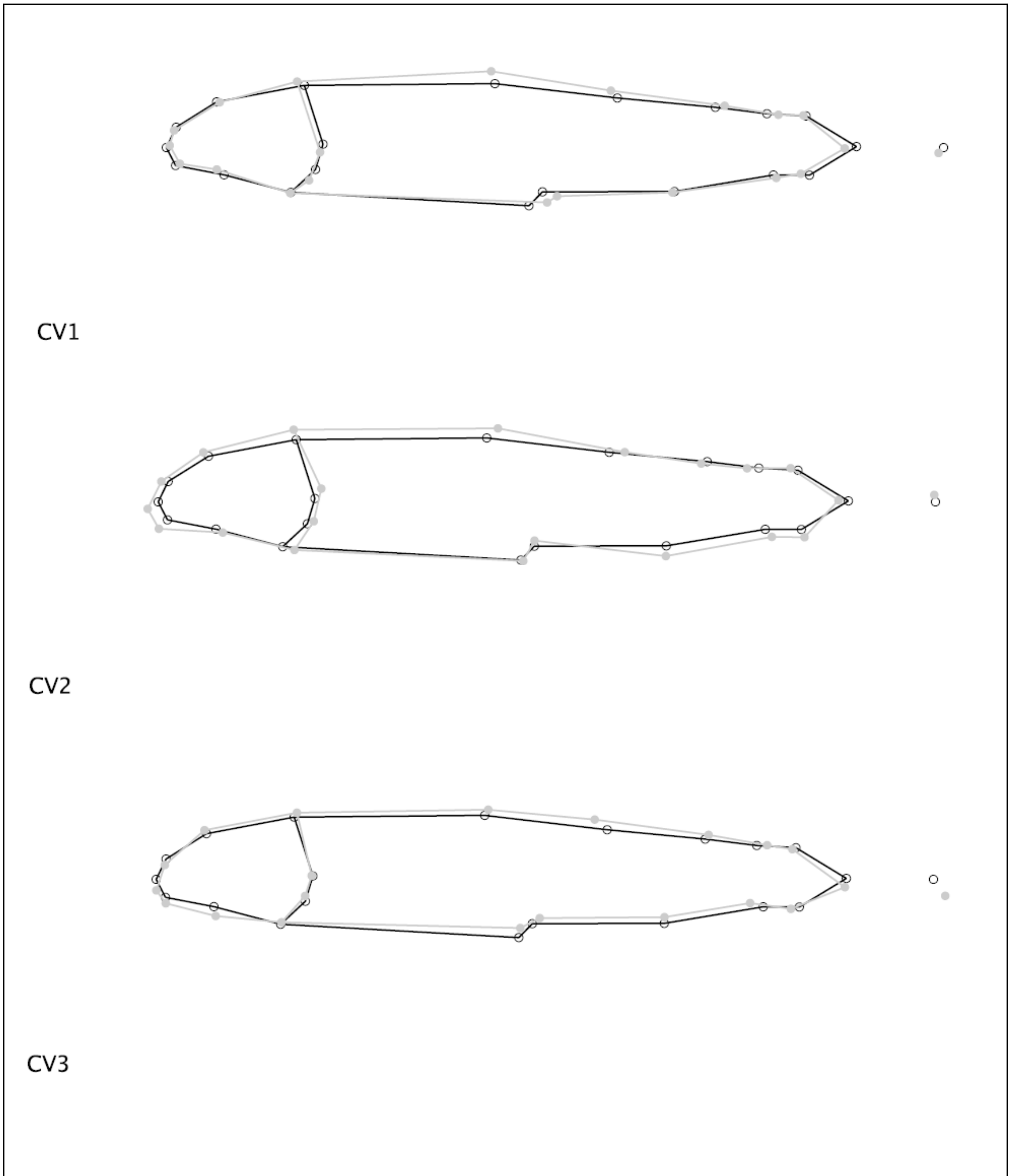

Figure 12: CV1 shows a little variability in body shape and CV2 shows variability in head shape between crosses

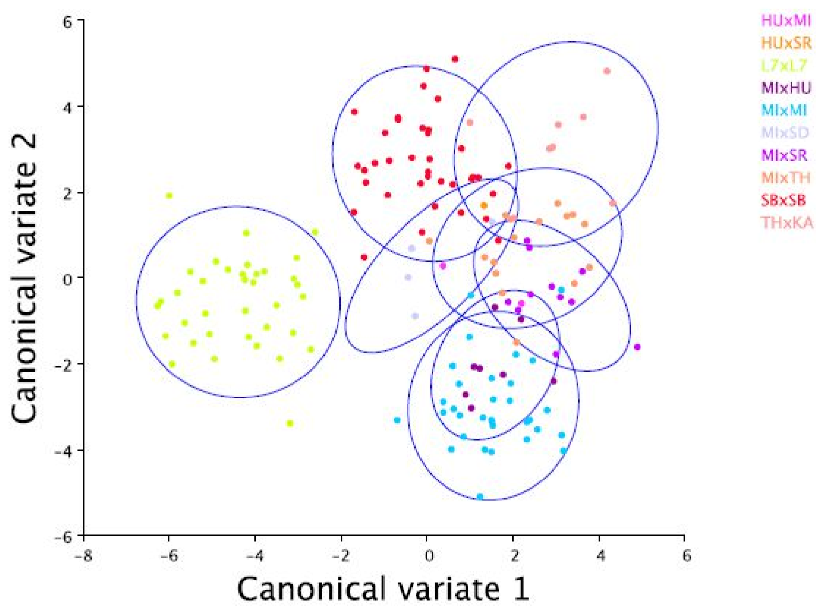

Figure 13: CV1 separates L7xL7 clearly from the rest. CV2 separates SBxSB and MIxMI from each other

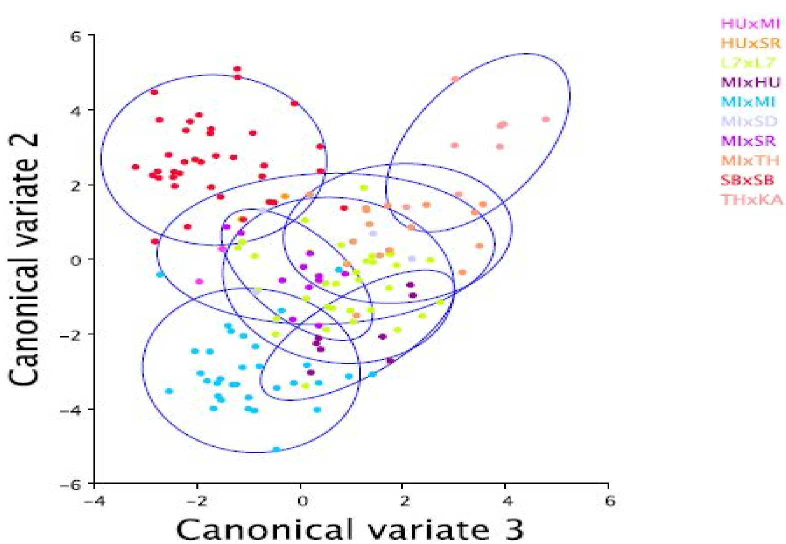

Figure 14: CV 3 separates THxKA partly from the rest.

The CVA for the crosses have one very major finding, that the pure SB crosses from TH, MI and L7 are very different from each other, while the hybrid crosses from the different combinations of locations appear to have more similar morphology to each other and intermediate between MIxMI and THxTH.

4 Discussion

One thing that seemed to have the most issue with the data was the fish bending in the pictures and the quality of the data suffers because of this fault. This is a problem in other similar studies and there are packages such as tpsutl that have and unbending function. One solution to this problem is to use those unbending programs and also to remove extreme samples (outliers) and test if there a morph effect to the bending by doing an ANOVA on PC1. There seemed to be no morph effect to the bending.

To answer the question if there is a significant difference in shape between morphs and hybrids from Þingvallavatn, the answer is yes. As can be seen in **table 9**, P-value between PlxSB and PLxSB and SB is <0.05. The most visible pattern observed is smaller heads and larger bodies *(see figure 9).* This is comparable to other studies (Skúlason et al., 1989). Both Kuðungableikja (LB) and Dvergbleikja (SB) are benthic and Sílableikja (PI) and Murta (PL) are pelagic (Skúlason et al., 1989). Their different feeding habit seems to be a logical explanation for the different shapes of morphs. The ecological niches that each morph keeps, has divided them physically.

To answer the second question, the answer is also yes, there is significant difference between the crosses. As can be seen from **table 11** and **table 13,** there are a few crosses which differ from each other significantly. It can also be seen in **figure 13** and **figure 14.** One thing of interest is that as can be seen from **figure 10**, there seems to be also some paternal effect in determination of the phenotype. Other studies have shown maternal effect on the offspring phenotype (Skúlason et all., 1989).

References

B. Jonsson and N. Jonsson. *(2001)* “Polymorphism and speciation in Arctic charr” *Journal of Fish* Biology 58: 605-638

B. Jonsson, S. Skúlason, S. S. Snorrason, O. T. Sandlund, H. J. Malmquist, P. M. Jónasson, R. Cydemo and T. Lindem. *(1988)* “Life history variation of polymorphic Arctic charr *(Salvelinus alpinus)* in Þingvallavatn, Iceland” *Canadian Journal of Fisheries and Aquatic Sciences 45(9):*1537-1547

Hrefna Sigurjónsdóttir and Karl *Gunnarson (1989) “Alternative* mating tactics of arctic charr *(Salvelinus alpinus)* in Þingvallavatn, Iceland*” Environmental Biology of fishes 26:* 159-176

Javier Negueruela Escudero*,(2011),* Variation in the D-loop of Arctic Charr (Salvelinus alpinus) morphs from Lake Thingvallavatn*, BS ritgerð, Líf og umhverfisvísindadeild, Háskóli Íslands,*

Kalina H. Kapralova, *(2014),* “Study of morphogenesis and miRNA expression associated with craniofacial diversity in Arctic charr *(Salvelinus alpinus)* morphs”*, PhD dissertation,*

Faculty of Life and Environmental Sciences, University of Iceland: 54-69

Odd Terje Sandlund, Karl Gunnarsson, Pétur M. Jónasson, Bror Jonsson, Torfinn Lindem, Kristinn P. Magnússon, Hilmar J. Malmquist, Hrefna Sigurjónsdóttir, Skúli Skúlason and Sigurður S. Snorrason. (1992) “The arctic charr *Salvelinus alpinus* in Þingvallavatn*” OIKOS 64:* 305-351

(no date)*Shape of Landmark Configurations: Procrustes Superimposition*

Sigurður S. Snorrason, Skúli Skúlason, Bror Jonsson, Hilmar J. Malmquist, Pétur M. Jónasson, Odd Terje Sandlund and Torfinn Lindem.*(1994) “*Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts“ *Biological Journal of the Linnean Socity 52:* 1-18

Skúli Skúlason, David L.G. Noakes and Sigurður S. Snorrason *(1989)*" Ontogeny of trophic morphology in four sympatric morphs of artic charr *Salvelinus alpinus* in Þingvallavatn, Iceland*" Biological Journal of the Linnean Socity 38*: 281-201

(no date)*Principal components: exploring the geometry of multidimensional morphospaces*

Tofallis, C. *(1999)*. "Model Building with Multiple Dependent Variables and Constraints" *J. R. Stat. Soc. D* **48** (3): 1–8