

**An Evaluation
of
Genetic Variation and
Reproductive Success
of Captive and Wild Woundfin
(*Plagopterus Argentissimus*)**



Study Number: DX-06-002
August 20, 2006



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FISH AND WILDLIFE SERVICE

Dexter National Fish Hatchery & Technology Center
P.O. Box 219, 7116 Hatchery Road
Dexter, NM 88230
(505) 734-5910 (505) 734-6130 FAX

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Submitted by: Molecular Ecology Program, Dexter National Fish Hatchery and Technology Center, Dexter, New Mexico 88230

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Photo courtesy of Steve Meisner

Project Summary

The woundfin (*Plagopterus argentissimus*) has been extirpated from most of the species' historic range, except for the mainstem Virgin River. This research was conducted to address the primary management question: are woundfin in the Virgin River genetically similar to captive stocks at Dexter. Our data was also able to determine if woundfin from Dexter are contributing to the recruitment in the Virgin River. These issues needed to be addressed as the wild population continued to decline, and the decision was made to supplement the wild fish with progeny from captive stocks at Dexter. In addition, fish were salvaged from drying reaches following several years of drought in the Virgin River, and a strategy was needed to incorporate the wild fish into the captive stock. Using 11 microsatellite

markers developed at Dexter National Fish Hatchery and Technology Center (Dexter), we evaluated genetic variation and reproductive success of woundfin in the Virgin River (wild) and in Dexter's long-term refuge stock (captive). Our data indicate that woundfin stocks at Dexter and in the Virgin River have similar high levels of genetic diversity, as reflected by heterozygosity values and allele numbers. The majority of genetic diversity occurs within stocks. The difference between extant stocks is very small, $F_{ST} = 0.005$. Although statistically significant, this F_{ST} value is typical of well mixed or undifferentiated stocks. Our results suggest that woundfin residing in the Virgin River and at Dexter are genetically similar and compatible for management purposes. The high degree of genetic similarity is probably the result of ongoing gene flow between captive and wild populations. The admixture analyses based on maximum likelihood and moment estimators suggest that Dexter's captive woundfin contributed to the recruitment in 2005 in the Virgin River at the rate of 48% in the area of the Quail Creek reservoir inflow, and 39% below the Washington Fields Diversion reach.

Introduction

The woundfin (*Plagopterus argentissimus* [Cope]) is a small, highly specialized, and essentially scaleless minnow (Cope 1874). Though early records indicate that woundfin was endemic to the lower Colorado River basin, including the Colorado River, Virgin River, Gila River, and their tributaries (Miller and Hubbs 1960; Deacon 1988; Williams 1995), current distribution is restricted to the upper Virgin River. Persistence of this species has partially been credited to active management by state and federal agencies, which include eliminating threats (predation and competition) from the nonnative red shiner (*Cyprinella lutrensis*) and minimizing the effects of water diversions (Steve Meisner personal communication).

The most immediate threat to woundfin has been the introduction of red shiner. Red shiner may impact survival of native fishes in the Virgin River through predation and competition for habitat and food resources (Addley and Hardy 1993). Since 1996, management efforts have focused on eradicating red shiner in the river and off-channel areas, and shiner-free reaches were created downstream of the Washington Fields Diversion to buffer sensitive upper basin fisheries (Fridell and Morvilius 2005).

A number of water diversion projects undertaken for agriculture and energy development, such as Quail Creek Reservoir in 1985, have decreased the abundance and distribution of woundfin (Fridell and Morvilius 2005). The Virgin River Resource Management and Recovery Program (Program) has been working to recover native species in the Virgin River, including woundfin and Virgin River chub (*Gila seminuda*). The habitat in the river above the Washington Fields Diversion serves as critical habitat for native fishes, and maintains the only viable populations of woundfin and Virgin River chub.

Woundfin from the Virgin River were brought to Dexter in 1979 to establish a captive refuge stock. Since then, the refuge population has been maintained at Dexter to offset potential catastrophic losses in the Virgin River (USFWS 1994). In response to low numbers of woundfin in the river above the Washington Fields Diversion, production from captive stocks at Dexter were stocked above Quail Creek and Ash Creek reaches in November of 2003 (Schijf et al. 2004) and 2004 (Fridell and Morvilius 2005).

Materials and Methods

Study Area

The Virgin River flows across the southern portion of Utah, through Washington County., and crosses into Arizona, and finally into Nevada before ending in Lake Mead. Critical habitat covers 80-90 miles of the Virgin River, but current woundfin distribution is confined to the upper 35-40 miles of watered stream (Steve Meisner, pers. comm.). A fish barrier was placed near the Arizona/ Utah border in the late 1980s to prevent upstream migration of non-native fish species (Steve Meisner, pers. comm.). Dexter records indicate over 39,000 woundfin from Dexter were stocked in the Virgin River between 1993 and 2000. These fish were released by the Nevada Department of Wildlife, and were stocked in Nevada, well below the fish barrier.

Sample collection

This study included a total of 418 woundfin samples (Table 1). Fin clips from the pelvic or caudal fins were taken from live fish in 2005 and 2006, except fish from Bel-DIV₀₁ were from samples preserved in 95% ethanol at Wahweap State Fish Hatchery, Utah. Bel-DIV₀₁ is comprised of fish salvaged during drought conditions in 2001 and brought to Wahweap. These fish subsequently perished and left no offspring. The Virgin River samples from below the Washington Fields Diversion (Bel-DIV₀₂₀₄) were salvaged in 2002 and 2004. These fish were brought to Dexter, quarantined, VIE tagged, and placed in the pond with the oldest group of woundfin Dex-YC_{<02}. The 2005 year classes sampled in the Virgin River were from various locations including below Washington Fields Diversion (Bel-DIV₀₅), immediately above the Washington Fields Diversion, the Virgin River immediately below the Quail Creek reservoir inflow, the Virgin River above Quail Creek reservoir inflow, and near the Ash Creek/LaVerkin Creek confluences with the Virgin River (Quail-Cr₀₅). Captive woundfin were sampled from four separate groups of woundfin maintained at Dexter. The first sample was from fish spawned before 2002 (Dex-YC_{<02}). The other samples were from the year classes: 2002 (Dex-YC₀₂), 2003 (Dex-YC₀₃), and 2005. 2005 samples from Dexter's captive stock were from the lot with mixed captive and wild ancestry (Dex-Sal₀₅) and a group with only captive parents (Dex-YC₀₅).

Table 1. Sample code, location, year class, and sample size of woundfin used in this study (woundfin spawned in 2004 by Dex-YC₀₂ and Dex-YC₀₃ were all returned to the Virgin River in November 2004).

Sample code	Location	Year class	Sample size
Bel-DIV ₀₁	Below the Washington Fields Diversion (salvaged)	2001	46
Bel-DIV ₀₂₀₄	Below the Washington Fields Diversion (salvaged)	2002/2004	46
Bel-DIV ₀₅	Below the Washington Fields Diversion	2005	48
Quail-Cr ₀₅	Near Quail Creek reservoir inflow	2005	41
Dex-Sal ₀₅	Dexter; offspring from Bel-DIV ₀₂₀₄ and Dex-YC _{<02}	2005	47
Dex-YC _{<02}	Dexter's mixed age broodstock	Before 2002	47
Dex-YC ₀₂	Dexter; offspring from Dex-YC _{<02}	2002	47
Dex-YC ₀₃	Dexter; offspring from Dex-YC _{<02} , and Dex-YC ₀₂	2003	48
Dex-YC ₀₅	Dexter; offspring from Dex-YC ₀₂ , and Dex-YC ₀₃	2005	48
Total			418

DNA extraction

A small piece of fin (10-20 mm²) was frozen at -78 °C, or air-dried and stored in a paper envelope. Genomic DNA was extracted from fins using a Qiagen Tissue Kit (Qiagen) as per extraction directions, and stored at -20 °C until needed.

PCR and microsatellite genotyping

Eleven of 12 polymorphic microsatellite markers developed at Dexter by Vu et al. (2005) were chosen for this ongoing population study: Par-B56MB, Par-C46TR, Par-B64ML, Par-B5T, ParC3TR, Par-B18, Par-D5BR, Par-B68, Par-C55BR, Par-B39, and Par-B3OT. The forward primer of each pair was labeled with a fluorescent phosphoramidite (6FAM, PET, NED, and VIC; Applied Biosystems). Microsatellite DNA was amplified via the polymerase chain reaction (PCR). Each 10 µl PCR contained: 20mM Tris-HCl, pH 8.4, 1.5mM MgCl₂, 0.2 mM dNTPs, 0.5 µM unlabeled reverse primers, 0.025 µM labeled forward primers, and 0.4 units Amplitaq Gold DNA polymerase (Applied Biosystems). Reaction mixtures were amplified using the following conditions: 96 °C for 9 min, then followed by 35-40 cycles of 95 °C for 40 sec, 52 °C for 1 min, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR products were visualized on an ABI 3100 Avant genetic analyzer. LIZ 500 (Applied Biosystems) was used as the size standard and composite genotypes for individual fish were compiled by scoring codominant alleles at each microsatellite locus using GeneMapper 3.5 software (Applied Biosystems).

Quality control Statistical analyses

ARLEQUIN 3.0 (Excoffier 2005) was used to identify deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992), observed (H_o) and expected (H_E) heterozygosities. We also used ARLEQUIN to calculate pairwise F_{ST} (Weir and Cockerham 1984) to assess population subdivision. Tests to determine the statistical significance of F_{ST} values used 10,100 computational permutations and sequential Bonferroni corrections (Holm 1979; Rice 1989). We used an analysis of molecular variation (AMOVA), based on F_{ST} distances, to describe the partitioning of molecular variation within and among samples.

Dex-Sal₀₅ represents a genetic combination from wild salvaged fish, and Dexter's captive broodstock. Samples from Dex-Sal₀₅ were analyzed and the contribution of the wild fish Bel-DIV₀₂₀₄ and captive stocks from Dexter Dex-YC_{<02} was calculated. This approach was then applied to samples from the Virgin River to calculate the relative contribution to recruitment in the Virgin River from Dexter's captive stocks. Based on records from VRRMRP and Dexter, captive woundfin had not been stocked in the Virgin River above the Washington Fields Diversion until July 2003. Bel-DIV₀₁ was the only sample from the Virgin River before the first stocking above the fish barrier, and we used this sample to represent the wild stock from the Virgin River. Dex-YC₀₃ was chosen to represent captive stocks because it was the primary captive contributor to the 2004 and 2005 YOY samples in the river (Bel-DIV₀₅ and Quail-Cr₀₅).

Using the assumed parental references, we employed LEADMIX 1.0 (Wang 2003) to determine the genetic contribution of wild and captive stocks in subsequent samples. LEADMIX provides three independent admixture estimators: one maximum likelihood (Wang 2003) and two moment estimators, one by Roberts and Hiorns (1965) and the other developed by Long (1991) and later elaborated by Chakraborty et al. (1992). Genetic drift was assumed to be 0.0001 for all three estimations. Finally, we conducted the same analyses on the progeny from mixed stocks at Dexter (Dex-Sal₀₅) using the known parental references, Dex-YC_{<02} and Bel-DIV₀₂₀₄.

Results

Patterns of microsatellite variation

The allele frequencies and number of alleles (N_A) were computed for each population and each locus (Appendix 1), and a range of 20 (Par-B68) to 34 (Par-D5BR) alleles was scored for each discrete locus. Woundfin at Dexter and in the Virgin River appeared to be genetically diverse, as reflected by both the allele number and heterozygosity rate (Table 2). The average number of alleles per locus was 15.64-20.09, and the expected heterozygosity was 0.89-0.92. Despite similar levels of heterozygosities, captive year classes contained the lowest number of alleles (15.6-18.8), compared to samples from the river (19.3-20.1), and offspring from the mixed broodstock Dexter and Salvage fish 2002 (19.0). Bel-DIV₀₁ had the greatest number of alleles (20.1), and Dex-YC₀₅ (15.6) possessed the fewest alleles. Further, we combined all captive year classes into a single captive population (Dex-YC_{all}) to compare with the wild population composed of all woundfin samples from the river (VR-YC_{all}) and the current population (VR-YC_{live}) in which Bel-DIV₀₁ was excluded. Similarly, VR-YC_{all} had the most alleles (24.8), followed by VR-YC_{live} (23.8), and Dex-YC_{all} (21.1).

Within the wild samples, VR-YC_{all} had the highest number of private alleles (37) not found in Dex-YC_{all}, or Dex-Sal₀₅. The extant wild samples (VR-YC_{live}) only had 5 private alleles,

Table 2. Mean values of observed heterozygosity (H_o), expected heterozygosity (H_E) and allele number (N_A) over 11 microsatellite loci, and total number of private alleles (N_{TP}) in each woundfin sample. N_s is the sample size from each group; Dex-YC_{all} (shaded) is all samples from Dex-YC_{<02}, Dex-YC₀₂, Dex-YC₀₃ and Dex-YC₀₅; VR-YC_{all} (shaded) includes all woundfin samples from the Virgin River (Bel-DIV₀₁, Bel-DIV₀₂₀₄, Bel-DIV₀₅, and Quail-Cr₀₅); VR-YC_{live} (shaded) includes all extant samples from the river (Bel-DIV₀₂₀₄, Bel-DIV₀₅, and Quail-Cr₀₅).

Sample	H_o	H_E	N_A	N_{TP}	N_s
Bel-DIV ₀₁	0.85	0.91	20.1	6	39-46

Bel-DIV ₀₂₀₄	0.90	0.91	19.6	2	41-46
Bel-DIV ₀₅	0.88	0.91	19.3	3	46-48
Quail-Cr ₀₅	0.90	0.92	19.7	4	35-41
VR-YC _{all}	0.88	0.92	24.8	37	171-181
VR-YC _{live}	0.89	0.92	23.8	5	129-135
Dex-Sal ₀₅	0.90	0.91	19.0	1	46-47
Dex-YC _{<02}	0.88	0.90	18.8	3	45-47
Dex-YC ₀₂	0.87	0.89	15.7	1	46-47
Dex-YC ₀₃	0.85	0.89	16.4	0	46-48
Dex-YC ₀₅	0.88	0.90	15.6	0	46-48
Dex-YC _{all}	0.87	0.91	21.1	6	185-190

Mean F-statistic values, which are collectively referred to as “inbreeding coefficients” included $F_{IS} = 0.027$, and $F_{IT} = 0.042$. F_{IS} is the measure of the relationship of heterozygosity in an individual, and the subpopulation. F_{IS} values in natural populations are usually very low, but within small populations the chances of mating between relatives increase, and F_{IS} can increase accordingly. F_{IT} is “the most inclusive measure of inbreeding in that it takes into account both the effects of nonrandom mating within subpopulations (F_{IS}) and the effects of population subdivision (F_{ST}) (Hartl and Clark 1989).”

Table 3. Pairwise F_{ST} values between woundfin samples. All captive stocks (Dex-YC_{all}), all wild (VR-YC_{all}), and extant wild stocks (VR-YC_{live}) are shaded. Asterisks indicate statistical significance of F_{ST} with p-value ≤ 0.01 (10,100 permutations; significance test for F_{ST} between individual stocks used sequential Bonferroni corrections).

	Bel-DIV ₀₂₀₄	Bel-DIV ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC _{<02}	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
Bel-DIV ₀₁	0.005	0.007*	0.003	0.008*	0.018*	0.021*	0.021*	0.016*
Bel-DIV ₀₂₀₄		0.005	-	0.002	0.004	0.010*	0.009*	0.008*
Bel-DIV ₀₅			0.003	0.007*	0.009*	0.013*	0.012*	0.009*
Quail-Cr ₀₅				-	0.005	0.006	0.003	0.006
Dex-Sal ₀₅					0.001	0.005	0.003	0.004
Dex-YC _{<02}						0.007*	0.004	0.007*
Dex-YC ₀₂							0.004	-
Dex-YC ₀₃								0.006

	Bel-DIV ₀₁	Bel-DIV ₀₂₀₄	Bel-DIV ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC _{<02}	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅	DX-YC _{all}
Bel-DIV ₀₁	0.005	0.007*	0.003	0.008*	0.018*	0.021*	0.021*	0.016*		
Bel-DIV ₀₂₀₄		0.005	-	0.002	0.004	0.010*	0.009*	0.008*		
Bel-DIV ₀₅			0.003	0.007*	0.009*	0.013*	0.012*	0.009*		
Quail-Cr ₀₅				-	0.005	0.006	0.003	0.006		
Dex-Sal ₀₅					0.001	0.005	0.003	0.004		
Dex-YC _{<02}						0.007*	0.004	0.007*		
Dex-YC ₀₂							0.004	-		
Dex-YC ₀₃								0.006		
VR-YC _{all}										0.008*
VR-YC _{live}										0.005*

Our data did not indicate substantial differences among woundfin samples. Pairwise F_{ST}

values ranged from 0 to 0.021 (Table 3). The wild sample Bel-DIV₀₁ appeared to be the most genetically distinct ($F_{ST} = 0.005-0.021$). The F_{ST} value between all the captive samples (DX-YC_{all}) and all the wild samples (VR-YC_{all}) was 0.008 and between all the captive and extant wild (VR-YC_{live}) samples was 0.005. Both measures, while small, were statistically significant. The results of AMOVA were comparable with the F-statistics analyses. The total variation based on the AMOVA measure within stocks was overwhelmingly high, 99.29%, while among-stock differences were significant, but small ($F_{ST} = 0.007$; p -value ≤ 0.0001 ; Table 4).

Table 4. Analysis of molecular variance (AMOVA) within and among woundfin samples/stocks using 10,100 permutations.

Source of	Sum of Variance	Percentage of variation	d. f.	squares	components	variation	Fixation index
P-value Among-stock	8 65.48	0.04	0.71	$F_{ST} = 0.0071$	≤ 0.0001	Within-stock	827 4063.98
Total	835 4129.46	4.95	100				4.91 99.29

Admixture and reproduction

We used LEADMIX to determine the genetic contribution of parental woundfin to subsequent year classes. We calculated the genetic contribution from the captive stock to the 2004 and the 2005 samples (Table 5). Genetic contribution to the recruitment in the Virgin River in 2005 from the captive stock was 44-54% in the sample Quail-Cr₀₅ and 37-44% in the Virgin River above the Washington Fields Water Diversion (Bel-DIV₀₅). The wild samples from 2002 and 2004 year classes (Bel-DIV₀₂₀₄) contained 31-48% genetic contribution from the captive stock. The three estimates of admixture from RH (Roberts and Hiorns 1965), LC (Long 1991; Chakraborty et al. 1992), and W (Wang 2003) showed agreement for most comparisons. Overall, the Quail-Cr₀₅ sample had the greatest genetic contribution from the captive stocks with an average of 48% over the three methods, compared to the other two wild samples, Bel-DIV₀₅ (39%) and Bel-DIV₀₂₀₄ (38%). LEADMIX suggests the captive 2005 offspring (Dex-Sal₀₅) were descended from wild and captive fish stocks at a rate of 55% from the captive broodstock (Dex-YC₋₀₂) and 45% from the wild salvaged fish (Bel-DIV₀₂₀₄).

Table 5. The degrees of admixture by the estimators of RH (Φ_{RH} ; Roberts and Hiorns 1965), LC (Φ_{LC} ; Long 1991; Chakraborty et al. 1992), and W (Φ_W ; Wang 2003). Parental references for the estimation of reproductive success for wild offspring samples used Bel-DIV₀₁ and Dex-YC₀₃; and parental references for Dex-Sal₀₅ (asterisk) used Bel-DIV₀₂₀₄ and Dex-YC₋₀₂. The 95% confidence intervals (CIs) for RH, LC, and W estimates are in parentheses, and were obtained from 1000 bootstrapping replicates over the 11 microsatellite loci. Φ_{Mean} is the mean value of the three estimations. Ns represents the sample size.

	Φ_{RH}	Φ_{LC}	Φ_W	$\Phi_{Mean} \pm SD$	Ns
Bel-DIV ₀₂₀₄	0.48 (0.37-0.57)	0.31 (0.20-0.45)	0.34 (0.14-0.68)	0.38 \pm 0.09	46
Bel-DIV ₀₅	0.44 (0.33-0.52)	0.37 (0.27-0.46)	0.37 (0.21-0.88)	0.39 \pm 0.04	48
Quail-Cr ₀₅	0.54 (0.43-0.63)	0.47 (0.42-0.52)	0.44 (0.31-0.59)	0.48 \pm 0.05	41
Dex-Sal ₀₅ *	0.56 (0.47-0.65)	0.51 (0.43-0.60)	0.58 (0.18-1.00)	0.55 \pm 0.04	47

Discussion

Genetic variation in woundfin

The measure of F_{ST} between sampled stocks suggests woundfin from Dexter and the Virgin River are similar to each other (Table 3). F_{ST} is a standard measure of how alike the individual subpopulation is to the total sampled population. Hartl and Clark (1997) explain F_{ST} as a measure that depicts the relationship between a theoretically homogenous group of organisms and the departure from that model in a group of subpopulations. Generally, populations that have undergone bottlenecks, founder effects, or fragmentation have higher F_{ST} values. An F_{ST} value of 0.007, although significant, (Table 4) is typical of well mixed or undifferentiated stocks, indicating very little difference exists between sampled woundfin stocks. This degree of variation is less than that found in the Rio

Grande silvery minnow ($F_{ST} = 0.018$; Osborne et al. 2005) that is considered a panmictic population for management purposes. Johnson et al. (1993) found similar low levels (0.002-0.006) in reef fish that appear to have no discernable population structure.

However, the overall F_{ST} value reported here of 0.005 was statistically significant, as were some pairwise comparisons. For example, all captive stocks including the oldest broodstock and the wild salvaged fish were statistically significantly different from the oldest wild stock, Bel-DIV₀₁. The mixed year class salvage fish Bel-DIV₀₂₀₄ were also different at a small but significant level from the later captive year classes Dex-YC₀₂, Dex-YC₀₃, and Dex-YC₀₅. These levels of difference, while significant, are believed to be low enough that it should not be of management concern except as a reminder that divergence can occur if no attempts are made to genetically manage the captive stocks. Comparing the broodstock Dex-YC_{<02} to their progeny Dex-YC₀₂ illustrates this quite well. The difference between these stocks is statistically significant with an F_{ST} value of 0.007. The overall pairwise F_{ST} value between all captive woundfin and Virgin River woundfin in this study (excluding the lost fish from Wahweap, Bel-DIV₀₁) is 0.005.

These low values are not typical of endangered species, for example Parker et al. (1999) found F_{ST} values ranging from 0.2 to 0.8 between populations of Gila topminnow. Habitat fragmentation and range reduction to small, isolated populations often results in population subdivision and an increase in F_{ST} in cutthroat trout ($F_{ST} = 0.12-0.32$; Wenburg et al. 1998; Taylor et al. 2003; Whiteley 2004). Cordes et al. found similar high values for Paiute cutthroat (*Oncorhynchus clarki seleniris*), a species with an extensive stocking history. F_{ST} values between populations of Paiute cutthroat ranged from 0.008-0.297 (Cordes et al. 2004). Smaller values were not significant, but most values above 0.09 were statistically significant. Mohave tui chub (*Siphateles bicolor mohavensis*) in Camp Cady have an F_{ST} value of 0.20 relative to the donor source population at Mohave Chub Spring, though these populations have only been isolated for about five decades (Chen et al. 2006b). F_{ST} values in our study were also less than those for steelhead presented by Heath et al. (2002). Heath et al. (2002) found F_{ST} values between populations averaged 0.05 or less, but the variation between populations was comparable to the variation between year classes. Their study found the variance between years was 2.27%, while the variance between populations was 2.14%. The results of our AMOVA analysis for woundfin indicate the total variation among samples is 0.71%, while within sample diversity is very high (99.29%).

Woundfin exhibit high levels of genetic variation, as reflected by heterozygosity rates and allele numbers. Though the captive and wild woundfin populations have a similar high level of heterozygosity (e.g. Dex-YC_{all}, 0.91; VR-YC_{all} and VR-YC_{live}, 0.92), the former has slightly fewer alleles (Dex-YC_{all}, 21.1) than the latter (VR-YC_{all}, 24.8; VR-YC_{live}, 23.8). The wild samples VR-YC_{all} had 37 private alleles, but when the Bel-Div01 sample was excluded, the remaining wild fish VR-YC_{live} had 5 private alleles, which was not very different than Dex-YC_{all}, which had 6 private alleles.

The average heterozygosity tends to increase with the allele number (Fig. 2). If divided into three categories based on the allele number, eight fishes have N_A below 5; five have N_A between 5 and 10; and three have N_A over 10. The woundfin ($N_A = 18.2$; $H_E = 0.90$) falls into Category III ($N_A > 10$; $H_E > 0.80$) with high genetic diversity comparable to two other warm water fishes, the Rio Grande silvery minnow (*Hybognathus amarus*) and Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis minonectes*). However, care must be taken with this comparison as it may overemphasize the levels of genetic diversity in woundfin compared to other species due to ascertainment bias.

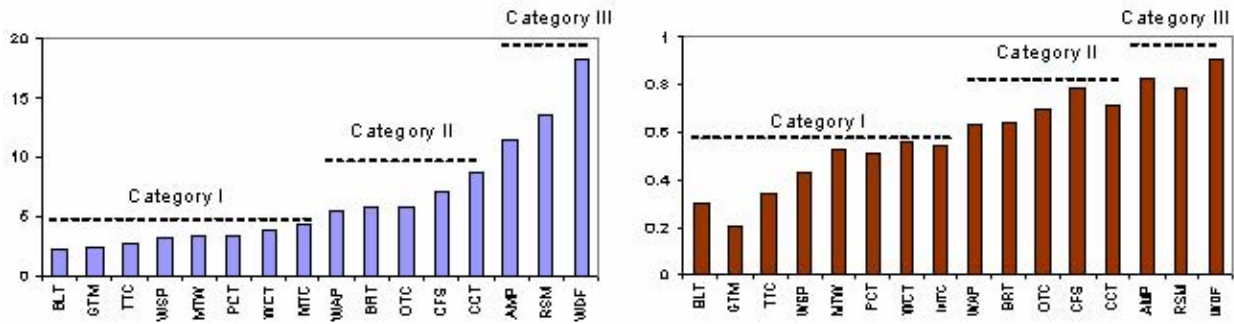


Fig. 2. Comparisons of average allele number (N_A left) and heterozygosity (H_e right) per microsatellite locus per sample among 16 freshwater fish species, including Bull trout (BLT; *Salvelinus confluentus*; Whiteley et al. 2004), Gila topminnow (GTM; *Poeciliopsis occidentalis occidentalis*; Parker et al. 1999), toikona tui chub (TTC; *Siphateles bicolor* spp; Chen et al. 2006a), White Sands pupfish (WSP; *Cyprinodon Tularosa*; Stockwell et al. 1998), Mountain whitefish (MTW; *Prosopium williamsoni*; Whiteley et al. 2004), Paiute cutthroat trout (PCT; *Oncorhynchus clarki seleniris*; Cordes et al. 2004), westslope cutthroat trout (WCT; *O. c. lewisii*; Taylor et al. 2003), Mohave tui chub (*S. b. mohavensis*; Chen et al. 2006b), Warm Spring pupfish (WAP; *C. nevadensis pectoralis*; Martin and Wilcox 2004), Brown trout (BRT; *Salmo trutta*; Jensen et al. 2005), Owens tui chub (OTC; *S. b. snyderi*; Chen et al. 2006a), Cape Fear shiner (CFS; *Notropis mekistocholas*; Gold et al. 2004), coastal cutthroat trout (CCT; *O. c. clarki*; Wenburg et al. 1998), Amargosa pupfish (AMP; *C. n. minonectes*; Martin and Wilcox 2004), Rio Grande silvery minnow (RSM; *Hybognathus amarus*; Osborne et al. 2005), and woundfin (DIV). The classification of three categories is based on the count of allele number (Category I, <5; Category II, 5-10; Category III, <10).

Ascertainment bias is the phenomenon where the number of alleles decreases proportionately with genetic distance from the species for which the markers were derived (Rogers and Jorde 1996; Hutter et al. 1998). Hutter et al. (1998) found “interspecific comparisons of microsatellite loci have repeatedly shown that the loci are longer and more variable in the species from which they are derived than are homologous loci in other species.” This finding is often compounded by the custom of identifying the longest clonal repetitive sequences and developing those markers selectively (Hutter et al. 1998).

A good example of ascertainment bias is found in the freshwater *Gila* complex. Microsatellite markers developed from bonytail chub (*Gila elegans*) DNA were used to screen humpback chub (*G. cypha*) (Keeler-Foster et al. 2004). Bonytail chub averaged 9 alleles across the 11 loci that amplified in both species, while humpback chub averaged 5 alleles at the same loci. Microsatellite markers used in this analysis are specific to the woundfin genome (Vu et al.

Reproductive success of woundfin

Our results indicate that captive and wild woundfin have contributed to woundfin recruitment in the Virgin River and at Dexter. Woundfin from the Dexter stock Dex-YC_{<02} and salvaged wild woundfin Bel-DIV₀₂₀₄ produced one of the 2005 year classes (Dex-Sal₀₅). This study indicates that Dex-Sal₀₅ has approximately 55% contribution from the captive parental stock Dex-YC_{<02} and 45% from the wild salvaged fish Bel-DIV₀₂₀₄. This example provides a controlled reference to the behavioral compatibility of these stocks. All captive stocks are kept in isolated ponds, and precise records kept of the numbers of individuals available to contribute to the next generation. The mixed group of broodstock that produced Dex-Sal₀₅ contained 199 captive woundfin from Dex-YC_{<02} and 101 from the Virgin River stock Bel-DIV₀₂₀₄. The numbers of potential captive parents (Dex-YC_{<02}) in the mixed broodstock was 66%, which is largely concordant with the mean estimate of parental contribution of 55% from captive parents using the genetic data.

Captive woundfin had not been stocked in the Virgin River above the Washington Fields Diversion until July 2003. However, 7,483 woundfin were stocked in the above the Washington Fields Diversion in 2003 (3,004) and in 2004 (4,479). We can hypothesize that samples collected from the river after 2003 would reflect shared ancestry with captive stocks from Dexter. It is important to note, however, that this analysis provides an estimate of the commonality of two groups, which we discuss as common ancestry, and neither the wild or captive stocks started as discrete, separate stocks. The captive stock originated from the Virgin River, and has been augmented from the river several times. Likewise, over

the years woundfin have been stocked into the Virgin River, so both stocks have had recent, ongoing gene flow. Given that caveat, our analysis indicates genetic contribution of captive stocks to wild stocks in the Quail-Cr₀₅ sample and in the Washington Fields Diversion (Bel-DIV₀₅) is about 48% and 39%, respectively. Bel-DIV₀₂₀₄ is comprised of fish salvaged below the Washington Fields Diversion in 2002 (86) and 2004 (88). These fish appear to contain 38% of the genetic background reflective of the captive stock at Dexter. These findings provide strong evidence that supplementations from Dexter's captive stocks in 2003 and 2004 have supplied genetic material to the wild population, as well as increased the number of woundfin in the Virgin River (Schijf et al. 2004; Fridell and Morvilius 2005).

Conservation and management strategies

Our research objective was to determine if woundfin in the Virgin River are genetically similar to captive stocks at Dexter and; based on our results, are woundfin from Dexter contributing to the recruitment in the Virgin River?

First, we examined genetic divergence between long time captive stocks at Dexter, and the wild population in the Virgin River. Domestication selection, genetic drift and founder effects are ways that the genetics of captive stocks may become different from the wild or donor stocks. Woundfin have been held at Dexter for almost three decades, but the captive stocks have received several augmentations from wild fish in the Virgin River. This gene flow acts as a mechanism to counter the effects of genetic drift, founder effects and domestication selection. Genetic drift, founder effects, and selection may act to create differences between populations, while gene flow acts to keep populations genetically similar (Whitlock 1992). Our results suggest woundfin stocks share a recent common ancestry and remain genetically similar to each other; captive and wild stocks are genetically compatible. Therefore, woundfin from the captive stocks at Dexter are genetically appropriate as a broodstock for augmentation purposes. Second, we addressed the question: are woundfin stocks from Dexter contributing to the recruitment in the Virgin River? Managers have many approaches to determining the success of an augmentation program, including mark-recapture analysis and repeated sampling strategies to contact marked fish. These efforts to evaluate the success of a stocking program can be problematic as fish may disperse to new locations, marks may be lost, and repeated electroshocking and seining can be detrimental to the habitat and fish. Genetic data can be used to determine the proportion of a population that share a common genetic background. We provide several statistical approaches to calculate the relative genetic components in captive stocks and wild stocks (Table 5). The results indicate that samples from 2006 in the Virgin River reflect from 38% to 48% captive genetic background. We also analyzed the progeny from a captive stock mixed with salvaged fish from the Virgin River to assess and verify the accuracy of our statistical approach. The 2005 year class Dex-Sal₀₅ that resulted from the mixed wild and captive stock at Dexter contained 45% of the wild genomic type and 55% of the captive genetic background, reflecting the expectation of random mating in the closed population.

Our results suggest the ongoing augmentation program in the Virgin River has been successful. Captive fish appear to be contributing to the Virgin River population with an increase in census numbers and through gene flow.

Management and recovery of woundfin have two primary components: maintenance and perpetuation of the captive stock at Dexter, and the management of the Virgin River population. Genetic tools allow managers to identify and mitigate potential sources of genetic risks. The research reported here suggests two assumptions are true. The captive population at Dexter and the remaining wild population are essentially one stock, and the augmentation program in the Virgin River is successfully integrating captive stocks into the Virgin River population.

Our management recommendations include:

Pooling multiple year classes of stock to prevent and mitigate the effects of temporal variation (Whitlock 1992). Pooled broodstocks can be split into two ponds to ensure a backup against a

catastrophic event at Dexter.

This program should continue to provide gene flow from the Virgin River into the captive stock. It is evident that ongoing gene flow between the captive stock and the Virgin River is bidirectional and successful in preventing the divergence of captive and wild stocks. We recommend that a regular infusion of wild fish every three years should provide adequate genetic material to prevent the future divergence of these stocks (Mills and Allendorf 1996). The number of wild individuals for augmenting captive stocks should be based on the VRRMRP committee's recommendation, but we recommend a minimum of 300 individuals every 3 years.

We also suggest the program should monitor the wild population by continuing to collect tissue samples during regular management activities. These samples could be collected and stored for genetic analysis every 5 years for comparison to this baseline.

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Appendix 1. Allele frequencies for the 11 microsatellite DNA loci examined in woundfin. Observed (H_o) and expected heterozygosities (H_e), p-value of Hardy-Weinberg equilibrium (P_{HW}) test, total number of alleles (N_A), and sample size (N_s) are summarized for each locus, with missing alleles indicated by "-". Asterisks indicate p-values ≤ 0.01 of Hardy-Weinberg equilibrium test.

Par-B56MB	Bel-WFD01	Bel-WFD0204	Bel-WFD05	Quail-Cr05	Dex-Sal05	Dex-YC<02	Dex-YC02	Dex-YC03	Dex-YC05
254	-	-	0.010	-	-	-	-	-	-
258	-	-	-	-	-	-	0.011	-	-
266	0.022	0.011	-	0.014	0.032	0.011	0.032	0.031	0.010
270	0.022	0.043	0.063	0.029	0.032	0.011	0.085	0.031	0.042
274	0.144	0.096	0.073	0.114	0.085	0.065	0.074	0.052	0.094
278	0.078	0.021	0.083	0.071	0.128	0.065	0.170	0.125	0.156
282	0.011	0.011	0.042	0.029	0.011	0.065	0.074	0.042	0.094
286	0.056	0.074	0.094	0.043	0.096	0.043	0.043	0.052	0.031
290	0.078	0.043	0.094	0.071	0.074	0.054	0.053	0.115	0.073
294	0.078	0.245	0.083	0.200	0.128	0.163	0.128	0.188	0.135
298	0.044	0.043	0.083	0.029	-	0.022	-	0.010	0.010
302	0.078	0.074	0.031	0.043	0.106	0.076	0.021	0.063	0.073
306	0.044	0.064	0.073	0.043	0.096	0.065	0.074	0.063	0.063
310	0.089	0.074	-	0.029	-	-	-	-	-
314	0.056	0.032	0.052	0.057	0.074	0.109	0.064	0.104	0.083
318	0.022	0.053	0.115	0.014	0.043	0.130	0.117	0.063	0.083
322	0.011	0.011	-	0.014	0.011	0.011	-	-	-
326	-	-	0.010	0.029	0.011	-	0.011	-	-
330	0.022	0.043	0.010	0.029	0.011	-	-	-	0.010
334	0.044	-	-	0.014	-	-	-	-	-
338	0.011	-	0.031	0.014	-	0.011	-	0.010	-
342	0.022	0.021	0.010	0.029	0.011	0.033	-	0.010	-
346	0.022	0.021	-	0.014	-	-	-	0.021	-
350	0.011	-	-	0.014	0.021	-	-	-	-
354	0.011	-	0.021	-	-	-	-	-	-

358	-	0.011	0.010	0.043	0.011	0.011	0.032	0.010	0.031
362	0.022	0.011	-	0.014	0.011	0.043	-	0.010	0.010
366	-	-	0.010	-	-	-	0.011	-	-
370	-	-	-	-	0.011	0.011	-	-	-
Ho	0.889	0.935	0.896	0.886	0.979	0.826	0.979	0.938	0.833
HE	0.943	0.911	0.936	0.934	0.924	0.924	0.915	0.913	0.918
P _{HW}	0.570	0.340	0.595	0.004*	0.362	0.075	0.718	0.605	0.221
N _A	23	20	20	24	20	19	16	18	16
N _S	45	47	48	35	47	46	47	48	48

Par-C46TR	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
117	0.011	-	-	-	-	-	0.021	-	-
123	-	0.011	0.021	-	0.011	-	-	-	-
125	0.022	0.011	0.021	0.061	0.033	0.021	-	0.021	0.021
127	0.076	0.011	-	-	0.022	-	-	-	-
129	0.043	0.021	0.021	0.037	0.033	0.021	0.043	0.031	0.073
131	0.011	0.011	0.010	0.024	-	-	-	-	0.010
133	-	0.011	0.010	-	-	-	0.011	-	0.010
135	0.011	0.011	0.021	-	0.022	0.011	0.011	0.010	0.010
137	0.011	-	-	0.012	-	-	0.011	-	-
139	0.011	0.043	0.073	0.024	0.130	0.085	0.074	0.083	0.052
141	0.011	0.011	-	-	0.011	-	0.011	-	0.031
143	0.011	0.064	0.063	0.049	0.087	0.032	0.181	0.115	0.125
145	0.076	0.011	0.063	0.073	0.054	0.011	0.064	0.031	0.063
147	0.120	0.149	0.240	0.110	0.087	0.223	0.096	0.188	0.135
149	0.087	0.106	0.104	0.061	0.130	0.160	0.053	0.073	0.094
151	0.163	0.096	0.104	0.171	0.109	0.085	0.106	0.156	0.063
153	0.087	0.096	0.042	0.110	0.065	0.128	0.128	0.073	0.146
155	0.109	0.085	0.104	0.049	0.022	0.021	-	0.021	-
157	0.043	0.074	0.042	0.049	0.054	0.096	0.096	0.073	0.052
159	0.033	0.032	0.031	0.061	0.033	0.053	0.011	0.031	0.010
161	0.022	0.074	-	-	0.054	-	0.011	0.021	0.031
163	0.033	0.053	0.021	0.085	0.022	0.032	0.053	0.042	0.052
165	-	-	-	0.012	-	-	-	-	-
167	-	0.021	0.010	0.012	0.022	0.011	0.021	0.031	0.021
169	-	-	-	-	-	0.011	-	-	-
173	0.011	-	-	-	-	-	-	-	-
Ho	0.891	0.957	0.917	0.780	0.935	0.957	0.936	0.854	0.938
HE	0.928	0.928	0.804	0.926	0.930	0.887	0.913	0.909	0.923
P _{HW}	0.583	0.498	0.573	0.001*	0.863	0.424	0.376	0.252	0.671
N _A	21	21	18	17	19	16	18	16	18
N _S	46	46	48	41	46	47	47	48	48

Par-B64ML	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
195	0.087	0.011	0.021	0.049	0.053	0.043	0.053	0.042	0.043
199	0.033	0.064	0.125	0.085	0.064	0.096	0.096	0.063	0.043
203	0.033	0.032	0.021	0.012	0.021	0.053	0.043	0.073	0.064
207	0.054	-	0.010	-	-	-	-	-	-
211	0.076	0.085	0.052	0.024	0.021	0.053	0.032	0.031	0.064
215	0.380	0.191	0.198	0.146	0.149	0.106	0.138	0.135	0.181
219	0.043	0.106	0.167	0.098	0.085	0.074	0.064	0.104	0.106

227	-	0.021	0.010	-	0.021	-	-	-	-
231	-	-	0.010	-	-	-	-	0.010	-
235	0.022	0.053	0.042	0.061	0.064	0.106	0.149	0.042	0.053
239	-	0.011	0.010	0.012	-	0.011	0.021	-	0.074
243	0.011	0.053	0.031	0.024	0.032	-	-	-	-
247	0.022	0.021	0.010	0.012	0.021	0.032	-	-	-
251	0.022	0.011	0.063	0.122	0.064	0.021	0.064	0.073	0.053
255	0.022	0.053	0.073	0.122	0.074	0.064	0.021	0.042	0.021
259	0.054	0.106	0.042	0.110	0.096	0.096	0.085	0.125	0.053
263	0.087	0.106	0.094	0.073	0.191	0.202	0.202	0.219	0.234
267	0.022	0.043	0.010	-	0.011	0.043	0.021	0.010	-
275	-	0.011	-	0.012	-	-	-	-	-
283	-	0.021	-	0.012	0.032	-	0.011	0.031	0.011
287	0.022	-	-	-	-	-	-	-	-
299	0.011	-	-	-	-	-	-	-	-
303	-	-	0.010	0.024	-	-	-	-	-
Ho	0.652	0.761	0.729	0.829	0.766	0.723	0.745	0.729	0.809
He	0.833	0.920	0.907	0.919	0.910	0.910	0.897	0.895	0.886
P _{HW}	0.000*	0.004*	0.008*	0.382	0.011	0.002*	0.046	0.041	0.161
NA	17	18	19	17	16	14	14	14	13
Ns	46	46	48	41	47	47	47	48	47

Par- B5T	Bel- WFD ₀₁	Bel- WFD ₀₂₀₄	Bel- WFD ₀₅	Quail- Cro ₅	Dex- Sal ₀₅	Dex- YC _{<02}	Dex- YC ₀₂	Dex- YC ₀₃	Dex- YC ₀₅
189	-	-	-	0.012	-	-	-	-	-
193	-	-	-	-	-	0.011	-	-	-
205	-	0.011	-	-	-	-	-	-	-
209	0.011	0.011	-	-	-	-	-	-	-
213	-	-	0.010	-	0.011	-	0.011	0.021	-
217	-	0.022	-	0.012	0.011	-	-	-	-
221	0.011	0.011	-	-	-	0.011	-	-	-
225	0.011	-	0.031	0.012	-	-	-	-	-
229	0.011	0.011	0.010	0.024	0.043	0.011	0.076	0.052	0.117
233	0.011	0.054	0.031	0.012	-	0.067	0.087	0.021	0.128
237	0.011	0.011	0.063	0.085	0.064	0.056	0.098	0.156	0.074
241	0.098	0.065	0.198	0.073	0.096	0.089	0.043	0.083	0.074
245	0.076	0.011	0.042	0.073	0.053	0.067	0.043	0.031	0.011
249	0.054	0.076	0.052	0.134	0.085	0.056	0.054	0.083	0.021
253	0.109	0.120	0.063	0.171	0.106	0.200	0.141	0.135	0.149
257	0.196	0.098	0.073	0.098	0.096	0.067	-	0.031	0.032
261	0.098	0.087	0.083	0.085	0.160	0.056	0.076	0.083	0.053
265	0.152	0.163	0.198	0.098	0.128	0.133	0.207	0.094	0.191
269	0.043	0.065	0.052	0.073	0.043	0.022	0.076	0.063	0.096
273	0.022	0.043	0.031	0.037	0.021	0.067	0.043	0.063	0.032
277	0.022	-	0.021	-	-	-	-	0.010	-
281	0.043	0.033	0.010	-	0.043	0.044	0.022	0.010	-
285	-	0.054	-	-	0.043	0.022	-	0.031	-
289	0.011	0.054	0.031	-	-	0.022	0.022	0.031	-
293	0.011	-	-	-	-	-	-	-	0.021
Ho	0.913	0.978	0.854	0.951	0.936	0.889	0.870	0.917	0.851
He	0.903	0.923	0.900	0.916	0.916	0.914	0.909	0.923	0.899
P _{HW}	0.480	0.774	0.262	0.534	0.733	0.555	0.415	0.757	0.391

NA	19	19	17	15	15	17	14	17	13
Ns	46	45	48	41	47	45	46	48	47

Par-C3TR	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
164	-	0.011	0.031	-	0.021	-	0.021	0.043	-
168	-	-	0.010	-	-	-	-	-	-
172	0.056	0.044	0.031	0.061	0.085	0.064	0.053	0.043	0.052
176	0.033	-	-	0.024	0.021	0.011	-	-	-
180	0.033	0.022	-	0.024	-	-	-	-	-
184	0.056	0.022	0.052	0.049	-	0.043	-	-	-
188	0.100	0.033	0.052	0.073	0.021	0.011	0.085	0.053	0.125
192	0.100	0.144	0.219	0.098	0.096	0.106	0.245	0.138	0.292
196	0.044	0.011	0.052	0.073	0.085	0.117	0.085	0.181	0.052
200	0.033	0.056	0.052	0.061	0.032	0.053	0.032	0.021	0.021
204	0.022	0.011	0.042	0.061	-	0.021	0.011	0.021	-
208	0.011	0.056	0.042	0.061	0.032	-	-	-	-
212	0.111	0.033	0.063	0.085	0.106	0.117	0.085	0.053	0.083
216	0.089	0.078	0.031	0.037	0.064	0.011	0.043	0.032	0.042
220	0.144	0.111	0.052	0.073	0.106	0.074	0.064	0.106	0.063
224	0.022	0.100	0.010	0.049	0.032	0.032	0.021	0.043	0.021
228	0.033	0.022	0.031	0.012	-	0.021	0.021	0.021	-
232	0.033	0.056	0.146	0.037	0.074	0.128	0.053	0.021	0.073
236	0.044	0.067	0.073	0.061	0.096	0.043	0.032	0.074	0.063
240	-	0.089	0.010	0.037	0.074	0.096	0.106	0.106	0.094
244	0.022	0.011	-	-	-	-	-	-	-
248	-	0.011	-	-	0.032	0.021	-	-	-
256	0.011	0.011	-	0.024	0.021	0.032	0.043	0.043	0.021
Ho	0.933	0.933	0.875	0.976	0.851	0.915	0.915	0.872	0.792
He	0.932	0.933	0.914	0.949	0.936	0.928	0.900	0.920	0.878
P _{HW}	0.290	0.377	0.356	0.649	0.445	0.209	0.378	0.793	0.024
NA	19	21	18	19	17	18	16	16	13
Ns	45	45	48	41	47	47	47	47	48

Par-B18	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
192	-	0.011	-	0.012	-	-	-	-	-
196	-	-	-	-	0.011	-	0.011	-	0.052
200	0.033	0.011	0.010	0.037	-	-	-	-	-
204	-	0.011	-	-	-	-	-	-	-
208	0.022	0.021	-	0.012	-	0.021	-	-	-
212	0.011	0.021	-	-	0.011	0.011	-	-	-
216	-	-	0.031	0.012	0.021	0.011	-	0.010	0.021
220	0.022	0.021	0.031	0.024	0.011	0.043	0.043	0.042	0.021
224	0.065	0.053	0.083	0.049	0.053	0.053	0.054	0.083	0.073
228	0.163	0.106	0.125	0.061	0.021	0.011	0.054	0.042	0.021
232	0.152	0.085	0.073	0.110	0.096	0.117	0.098	0.083	0.094
236	0.043	0.085	0.115	0.073	0.170	0.191	0.196	0.240	0.125
240	0.152	0.074	0.146	0.171	0.128	0.085	0.130	0.094	0.125
244	0.065	0.074	0.083	0.110	0.085	0.096	0.130	0.063	0.135
248	0.076	0.043	0.042	0.024	0.053	0.011	-	-	0.021
252	0.054	0.106	0.083	0.122	0.106	0.128	0.065	0.125	0.063

256	0.065	0.170	0.104	0.098	0.149	0.149	0.098	0.094	0.135
260	0.011	0.021	0.010	0.024	0.011	0.021	0.076	0.052	0.073
264	0.011	0.021	0.031	0.012	-	0.021	0.022	0.010	-
268	-	0.043	0.031	0.024	0.043	0.011	-	0.052	0.031
276	0.011	-	-	-	-	-	0.022	-	0.010
280	0.033	-	-	0.012	0.011	0.011	-	0.010	-
284	0.011	0.011	-	-	-	0.011	-	-	-
292	-	0.011	-	0.012	0.011	-	-	-	-
296	-	-	-	-	0.011	-	-	-	-
Ho	0.739	0.848	0.917	0.951	0.851	0.809	0.913	0.854	0.917
He	0.915	0.928	0.917	0.917	0.905	0.900	0.903	0.892	0.915
P _{HW}	0.003*	0.182	0.664	0.858	0.039	0.119	0.129	0.514	0.545
N _A	18	20	15	19	18	18	13	14	15
N _S	46	46	48	41	47	47	46	48	48

Par-D5BR	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC _{<02}	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
249	-	0.011	-	-	0.021	-	-	-	-
253	-	-	-	0.012	-	-	-	-	-
257	0.012	0.043	0.010	0.012	0.021	0.033	0.011	0.031	-
261	0.012	0.053	0.010	0.012	0.032	0.033	-	0.021	0.010
265	-	-	-	-	0.011	0.022	-	-	-
269	0.012	-	-	-	-	-	-	-	-
273	0.012	0.043	0.031	0.012	0.021	0.033	0.032	0.083	0.063
277	-	0.021	0.021	-	0.043	0.011	0.011	0.031	0.031
281	0.023	0.011	0.021	0.012	0.064	0.011	0.011	-	0.010
285	0.081	0.043	0.021	0.061	0.064	0.044	0.074	0.177	0.094
289	0.035	0.021	0.031	0.037	0.043	0.011	0.032	0.031	0.063
293	0.023	0.053	0.021	0.024	0.074	0.067	0.053	0.010	0.083
297	0.035	0.011	0.063	0.012	0.043	0.111	0.043	0.073	0.052
301	0.105	0.096	0.177	0.061	0.043	0.044	0.064	0.052	0.125
305	0.047	0.011	0.031	0.073	0.021	0.011	0.032	-	0.021
309	0.047	0.032	0.031	0.098	0.053	0.033	0.149	0.052	0.083
313	0.058	0.032	0.021	0.049	0.011	0.011	0.053	0.042	0.010
317	0.081	0.032	0.115	0.061	0.021	0.033	0.021	0.031	0.052
321	0.035	0.106	0.031	0.024	0.064	0.089	-	0.010	0.010
325	0.047	0.043	0.052	0.061	0.032	0.067	0.053	0.073	0.083
329	0.116	0.106	0.115	0.146	0.064	0.089	0.202	0.188	0.115
333	0.058	0.106	0.010	0.012	0.064	0.044	0.053	0.021	0.031
337	0.023	-	0.010	0.061	0.032	0.044	0.074	0.031	0.052
341	0.012	0.021	0.021	0.061	0.064	0.056	0.021	-	0.010
345	0.070	-	0.031	0.012	0.021	0.022	-	0.010	-
349	0.012	-	0.031	0.012	0.011	0.011	-	-	-
353	0.012	0.043	0.021	0.024	0.011	0.022	-	0.010	-
357	-	0.021	-	0.024	0.011	0.011	0.011	0.021	-
361	0.023	0.021	0.031	0.012	0.032	0.011	-	-	-
365	-	0.011	-	-	0.011	-	-	-	-
369	-	0.011	0.021	-	-	0.011	-	-	-
373	0.012	-	-	-	-	0.011	-	-	-
425	-	-	0.010	0.012	-	-	-	-	-
437	-	-	0.010	-	-	-	-	-	-
Ho	0.860	0.957	0.979	0.927	0.915	0.889	0.979	0.833	0.958
He	0.950	0.949	0.934	0.946	0.963	0.958	0.914	0.912	0.932

P _{HW}	0.397	0.191	0.984	0.084	0.228	0.049	0.789	0.148	0.805
N _A	25	25	27	26	28	28	19	20	19
N _S	43	46	48	41	47	45	47	48	48

Par-B68	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
271	0.011	0.012	-	-	-	-	-	-	-
275	0.023	0.024	-	-	-	-	-	-	-
283	-	-	-	0.012	-	-	-	-	-
291	0.034	0.012	-	-	0.011	0.011	-	-	-
295	0.034	0.036	0.021	0.012	0.053	0.074	0.032	0.031	0.022
299	0.057	0.024	0.052	0.049	0.021	0.032	0.032	0.031	0.011
303	0.023	0.048	0.042	0.012	0.074	-	0.011	0.010	0.054
307	0.170	0.071	0.135	0.098	0.117	0.106	0.106	0.042	0.130
311	0.193	0.167	0.177	0.195	0.181	0.138	0.181	0.177	0.163
315	0.091	0.274	0.167	0.244	0.191	0.213	0.330	0.292	0.293
319	0.045	0.048	0.083	0.085	0.106	0.053	0.085	0.063	0.098
323	0.091	0.036	0.073	0.061	0.032	0.053	0.011	0.021	0.022
327	0.057	0.083	0.042	0.073	0.032	0.074	0.053	0.094	0.011
331	0.023	-	0.031	0.024	0.053	0.021	0.011	0.063	0.022
335	0.045	0.060	0.125	0.073	0.074	0.181	0.074	0.135	0.087
339	0.080	0.071	0.031	0.024	0.032	0.021	0.053	0.010	0.043
343	0.011	-	0.010	0.024	0.021	0.011	-	0.010	0.011
347	0.011	0.024	0.010	-	-	-	-	-	-
351	-	0.012	-	0.012	-	-	-	-	-
355	-	-	-	-	-	0.011	0.021	0.021	0.033
Ho	0.909	0.829	0.833	0.902	0.872	0.872	0.745	0.792	0.804
He	0.906	0.886	0.895	0.877	0.898	0.882	0.836	0.859	0.857
P _{HW}	0.687	0.104	0.401	0.163	0.510	0.828	0.486	0.101	0.400
N _A	17	16	14	15	14	14	13	14	14
N _S	44	41	48	41	47	47	47	48	46

Par-C55BR	Bel-WFD ₀₁	Bel-WFD ₀₂₀₄	Bel-WFD ₀₅	Quail-Cr ₀₅	Dex-Sal ₀₅	Dex-YC< ₀₂	Dex-YC ₀₂	Dex-YC ₀₃	Dex-YC ₀₅
192	-	-	-	0.013	0.011	-	-	-	-
196	0.011	-	0.032	-	-	-	-	-	-
200	0.011	-	0.011	-	-	0.011	-	-	-
212	0.022	0.065	-	0.013	0.022	0.011	-	-	0.010
216	0.011	-	-	-	0.011	0.011	-	-	-
220	0.033	0.011	0.021	-	-	0.022	-	-	-
224	0.011	0.011	0.032	0.050	0.076	0.022	0.106	0.115	0.115
228	0.076	-	0.064	0.038	0.054	-	0.021	0.094	0.052
232	0.076	0.109	0.053	0.088	0.033	0.109	0.032	0.135	0.052
236	0.043	0.043	0.043	0.038	0.022	0.065	0.074	0.021	0.031
240	0.065	0.033	0.064	0.100	0.022	0.022	0.106	0.021	0.042
244	0.033	0.065	0.043	0.050	0.043	0.033	0.011	0.052	0.031
248	0.076	0.054	0.064	0.025	0.011	0.043	0.011	0.010	-
252	0.098	0.098	0.138	0.100	0.120	0.098	0.117	0.177	0.083
256	0.054	0.076	0.117	0.063	0.141	0.130	0.149	0.104	0.125
260	0.054	0.109	0.085	0.088	0.076	0.087	0.149	0.063	0.115
264	0.109	0.163	0.053	0.163	0.174	0.196	0.096	0.094	0.177
268	0.054	0.076	0.043	0.063	0.076	0.065	0.021	0.052	0.083

272	0.043	0.033	0.021	0.025	0.011	0.011	0.021	0.010	-
276	0.054	0.022	0.043	0.013	0.022	-	0.011	-	-
280	-	-	0.043	-	0.011	-	-	-	-
284	0.054	0.011	0.021	0.013	0.022	0.011	-	0.010	0.010
288	-	0.011	-	0.025	0.011	-	-	-	0.010
292	-	0.011	0.011	0.025	0.033	0.054	0.074	0.042	0.063
296	0.011	-	-	0.013	-	-	-	-	-
Ho	0.935	0.867	0.894	0.900	0.870	0.870	0.936	0.813	0.896
He	0.946	0.925	0.941	0.931	0.918	0.909	0.906	0.909	0.909
P _{HW}	0.095	0.203	0.052	0.069	0.410	0.106	0.923	0.075	0.331
N _A	21	18	20	20	21	18	15	15	15
N _S	46	45	47	40	46	46	47	48	48

Par- B39	Bel- WFD ₀₁	Bel- WFD ₀₂₀₄	Bel- WFD ₀₅	Quail- Cr ₀₅	Dex- Sal ₀₅	Dex- YC< ₀₂	Dex- YC ₀₂	Dex- YC ₀₃	Dex- YC ₀₅
306	0.011	-	-	-	-	-	-	-	-
318	0.011	-	0.010	0.012	-	0.011	-	0.042	-
322	-	-	0.021	0.012	-	-	-	-	-
326	-	0.021	0.021	0.037	0.064	0.106	0.064	0.052	0.042
330	-	-	0.010	0.037	0.011	0.043	-	0.031	-
334	0.033	0.043	0.031	0.049	0.053	-	0.032	0.010	0.010
338	0.011	0.043	0.063	0.024	-	0.053	0.032	0.042	0.073
342	0.043	0.053	0.073	0.037	0.043	0.043	0.011	0.052	0.031
346	0.022	0.074	0.063	0.049	0.032	0.032	0.043	0.010	0.031
348	-	0.011	-	-	-	0.011	-	0.010	0.010
350	0.076	0.085	0.042	0.073	0.032	0.053	0.011	0.021	0.031
354	0.043	0.053	0.073	0.049	0.021	0.011	0.064	0.031	0.073
358	0.141	0.085	0.094	0.098	0.117	0.085	0.181	0.094	0.115
362	0.098	0.170	0.083	0.134	0.202	0.202	0.277	0.229	0.125
366	0.141	0.074	0.094	0.134	0.117	0.043	0.053	0.104	0.125
370	0.087	0.074	0.052	0.049	0.043	0.053	0.053	0.021	0.042
374	0.054	0.096	0.115	0.049	0.064	0.053	0.032	0.021	0.094
378	0.022	0.011	0.031	0.061	0.085	0.085	0.096	0.135	0.115
382	0.076	0.043	0.042	0.024	0.053	0.011	0.032	-	0.021
386	0.065	0.011	0.021	0.012	0.032	0.021	0.011	0.031	0.042
390	0.033	0.011	0.021	-	-	-	-	-	-
394	-	0.021	-	-	-	0.011	-	-	-
398	0.022	-	-	0.024	-	0.021	-	-	-
402	0.011	0.021	0.042	0.024	0.021	0.053	-	0.042	0.010
406	-	-	-	0.012	0.011	-	0.011	0.021	0.010
Ho	0.826	0.913	0.938	0.878	0.936	0.915	0.830	0.833	0.958
He	0.926	0.928	0.942	0.941	0.913	0.923	0.872	0.903	0.926
P _{HW}	0.079	0.554	0.803	0.003*	0.566	0.613	0.012	0.368	0.776
N _A	19	19	20	21	17	20	16	19	18
N _S	46	46	48	41	47	47	47	48	48

Par- B3OT	Bel- WFD ₀₁	Bel- WFD ₀₂₀₄	Bel- WFD ₀₅	Quail- Cr ₀₅	Dex- Sal ₀₅	Dex- YC< ₀₂	Dex- YC ₀₂	Dex- YC ₀₃	Dex- YC ₀₅
172	0.154	0.120	0.076	0.037	0.043	0.044	0.117	0.033	0.106
176	0.026	0.011	0.022	0.012	0.022	0.011	0.011	-	-
180	0.051	0.011	0.011	-	0.011	0.022	-	-	-
184	0.026	-	0.011	0.024	0.011	0.011	-	-	-

188	0.013	0.098	0.011	0.037	0.033	0.022	0.085	0.076	0.138
192	-	0.022	0.011	0.037	0.011	0.011	-	-	-
196	0.026	0.011	0.022	0.024	-	0.022	0.011	0.033	0.021
206	-	-	-	-	-	0.011	-	-	-
210	-	0.011	-	0.012	0.022	-	-	-	-
214	0.013	-	0.065	0.049	0.043	0.022	0.011	0.022	0.011
218	0.013	0.022	0.022	0.024	0.033	0.044	0.043	-	0.053
222	0.013	0.043	0.054	0.024	0.022	0.156	0.053	0.076	0.032
226	0.064	0.022	0.033	0.024	0.065	0.111	0.053	0.011	0.085
230	0.026	-	0.109	0.049	0.054	0.033	0.043	0.054	0.053
234	0.051	0.043	0.022	0.037	0.022	0.022	-	-	0.011
238	0.038	0.065	0.043	0.012	0.011	0.022	0.021	-	0.021
242	0.064	0.098	0.098	0.098	0.043	0.044	0.074	0.120	0.021
246	0.051	0.054	0.043	0.098	0.054	0.089	0.043	0.141	0.064
250	0.038	0.065	-	0.061	0.087	0.067	0.053	0.054	0.053
254	0.103	0.065	0.011	0.037	0.130	0.089	0.117	0.054	0.128
258	0.077	0.076	0.130	0.049	0.076	0.011	0.032	0.065	0.043
262	0.077	0.065	0.065	0.110	0.109	0.033	0.064	0.087	0.074
266	0.026	0.033	0.054	0.061	0.011	0.011	-	0.011	-
270	-	0.065	0.033	0.024	0.054	0.067	0.053	0.087	0.032
274	0.013	-	0.033	0.037	0.011	0.011	0.064	0.065	0.053
278	0.038	-	-	-	-	-	-	-	-
282	-	-	0.011	-	0.022	0.011	0.053	0.011	-
294	-	-	0.011	0.024	-	-	-	-	-
Ho	0.821	0.956	0.848	0.927	0.957	1.000	0.915	0.913	0.936
He	0.946	0.940	0.943	0.955	0.945	0.937	0.940	0.930	0.930
P _{HW}	0.000*	0.359	0.401	0.800	0.938	0.744	0.885	0.090	0.728
NA	22	20	24	24	24	25	19	17	18
Ns	39	45	46	41	46	45	47	46	47