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Estimates of genetic variation among Midwestern populations of Ascaris suum using RAPD fingerprinting

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Estimates of Genetic Variation Among Midwestern Populations of *Ascaris suum* Using RAPD Fingerprinting

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ABSTRACT:

The objective of this research is to characterize the genetic differentiation of several midwestern geographic populations of Ascaris suum, and to understand if this genetic variation is due to gene flow resulting from the transport of pigs or if, instead, isolated "island" populations undergo independent differentiation. The method of data acquisition was Random Amplified Polymorphic DNA (RAPD) fingerprinting. With this method, an oligonucleotide primer is used to amplify regions of genomic DNA; the amplified products are separated by agarose gel electrophoresis and visualized by staining. The polymorphisms were scored and analyzed using the Hardy-Weinberg equation and F statistics. The data indicates that there is great genetic differentiation and therefore little gene flow among the seven A. suum populations. Further research with the use of isozyme and RFLP techniques and larger population sizes will allow for more complete analysis of the genetic variability.

Key Words: Ascaris suum, genetic variability, polymorphism, RAPD

In the past, approaches to estimate genetic variation and gene flow consisted primarily of two methods- restriction fragment length polymorphism (RFLP) and isozyme polymorphism. The RFLP technique uses a restriction enzyme to cut DNA at specific recognition sites. The fragments generated are separated by size on an agarose gel and detected with specific DNA probes to reveal restriction fragment patterns. The polymorphic, or variable, bands are then analyzed for all the individuals of the population sample to determine genetic differentiation. The RFLP method has several drawbacks in that it requires large quantities of relatively pure DNA, specific probes, and use of radioactive materials (Chalmers et al., 1992). Analysis of the generated data is often a tedious process. Also, small changes in nucleic acid sequence within the generated fragment may go undetected and therefore, will not be counted as a genetic variation.

The isozyme technique uses electrophoresis to separate protein molecules on the basis of their net charge. Thus, different migration distances reflect different amino acid sequences, and in turn, differences in the encoding nucleic acid sequences. These genetic differences constitute different alleles of the protein locus. Comparison of the allelic variation of individuals in a population allows for analysis of the genetic variation. The isozyme technique has a drawback in that the analysis is often based on a relatively small number of protein loci, thus potentially resulting in a misleading estimate of variation, since some enzymatic loci will always display low variability (Nadler, 1990). Also, nucleic acid changes which do not alter the amino

acid makeup of the protein go undetected by the isozyme method, although these changes do reflect actual genetic variation.

A new technique is the use of the random amplified polymorphic DNA (RAPD) fingerprinting to detect polymorphisms at the nucleic acid level. The technique is based on a process in which a short oligonucleotide primer of random (but known) sequence binds to complementary sequences on separated template DNA strands and undergoes extension. The resulting strands, consisting of one old strand and one newly formed strand, reanneal and repeat the entire process over, thus increasing the amount of DNA at an exponential rate with each cycle.

The DNA fragments which result from amplification constitute the bands seen when the DNA is separated by agarose gel electrophoresis and stained with ethidium bromide. The presence or absence of a particular size band among individuals represents polymorphism at that locus. RAPD products are usually dominant markers (Williams et al., 1990; Devos & Gale, 1992), and thus homozygotes and heterozygotes cannot be distinguished. The absence of a band is a homozygous recessive marker.

The advantage of the RAPD method is that it does not require any knowledge of the template DNA sequence. Because the analysis is at the nucleic acid level, polymorphisms which result from nucleotide substitutions, deletions, or insertions, or from changes in the location of the priming site can be detected (Williams et al., 1990). Thus, this method is a relatively sensitive estimate of genetic variation. In addition, the RAPD approach can be used

for locating disease resistant genes, identifying chromosome-specific markers, and for identification of strains and varieties.

The study at hand seeks to characterize the genetic differentiation of several geographic populations of Ascaris suum (a parasitic nematode of pigs), and to understand if this variation is due to gene flow resulting from the transport of pigs across farms or if isolated "island" populations of Ascaris suum undergo independent genetic differentiation. Polymorphisms of Ascaris can be generated by any of the three techniques discussed previously; RAPD fingerprinting was chosen because it has the potential to yield a large number of polymorphic markers.

The master mix for the RAPD reactions consisted of water, 10X reaction buffer, DMSO, each of the four deoxynucleotides, the primer, and TAQ polymerase. Magnesium-free 10X reaction buffer was substituted for standard 10X reaction buffer (1.5mM $MgCl_2$) and supplemental magnesium chloride was added to a final concentration of 2.0mM; this reaction mix generated brighter and better-resolved bands. Twenty-four microliters of mix were distributed into individual tubes, to which one microliter of DNA template (75ng) was added. A mineral oil overlay prevented evaporation of the liquid. The tubes were placed in a thermocycler and underwent timed temperature settings corresponding to denaturation of the DNA template strands, annealing of the primer, and extension of the annealed primer. The thermocycler repeated the cycle 45 times, thus generating approximately 3.5×10^{13} new copies of the DNA

fragment. The DNA was then electrophoresed in a 1.2% agarose gel containing 1X TBE buffer, and stained with ethidium bromide.

At first, several primers were surveyed to find those which produced good scorable polymorphic bands. Using these, all the individual Ascaris suum were tested and scored. Scoring consisted of noting absence or presence (0 and 1, respectively). Primers produced from one to three scorable polymorphic bands; some produced monomorphic bands which were also scored. In all, 18 polymorphic markers were obtained from 9 different primers.

Absence of a band was assumed to be a homozygous recessive genotype at that locus, or equal to q^2 (recessive genotype frequency) in the Hardy-Weinberg equation. q is the frequency of the recessive allele and was determined by taking the square root of q^2. p, the frequency of the dominant allele, was determined from the equation $p+q=1$; p^2 is the frequency of the homozygous dominant genotype. Note, however, that the homozygous dominant genotype and the heterozygous genotype are based on the calculation of the recessive genotype and therefore do not represent the observed value which might be obtained if heterozygosity could be determined using RAPD markers.

Seven populations of Ascaris suum from five midwestern localities were analyzed (the Cassopolis and Burlington localities each contained two pigs and therefore two Ascaris suum populations) based on F-statistics generated from the allele and genotype frequencies, using the Qubecalc computer program. Results are tabulated in Tables 1-4: 1) all seven populations, 2) all five

localities (by combination of the two populations in Cassopolis and in Burlington into one population for each), 3) the two populations located within the Cassopolis locality, and 4) the two populations located within the Burlington locality. H_O is the expected random mating heterozygosity of an individual, H_S is the expected heterozygosity of an individual in an equivalent random mating subpopulation, and H_T is the expected heterozygosity of an individual in an equivalent random mating total population. These H statistics are used to generate the F statistics (Hartl, 1988). F_{IS} is the inbreeding coefficient, or the reduction in heterozygosity of an individual due to nonrandom mating within its subpopulation. F_{ST} is the fixation index, or the reduction in heterozygosity of a subpopulation due to random genetic drift. F_{IT} is the overall inbreeding coefficient of an individual due to nonrandom mating and to random genetic drift, or the reduction in heterozygosity of an individual relative to the total population.

For the purposes of this study, only the F_{ST} statistic was considered in the analysis of the data since its calculation does not depend on the frequency of observed heterozygosity (a value which cannot be obtained using RAPD technique). The value of F_{ST} ranges from zero (no genetic differentiation) to one (complete fixation for alternative alleles). A very general guideline for interpretation of F_{ST} is as follows: 0.00- 0.05 indicates little genetic differentiation, 0.05- 0.15 indicates moderate genetic differentiation, 0.15- 0.25 indicates great genetic

differentiation, and greater than 0.25 indicates very great genetic differentiation (Hartl, 1988).

According to the above interpretation, there is great genetic differentiation (23.5%) among the seven populations (a population being all the Ascaris suum individuals from an individual pig). The rest of the genetic variation (76.5%) is found within the populations themselves. Among the five localities, there is moderate genetic differentiation (15.6%), whereas 84.4% of the genetic differentiation is located within the localities. Of the two localities which each contained two populations of Ascaris suum, the Cassopolis locality displays little genetic differentiation, 5.2%, among its populations and 94.8% of the variation within the populations themselves. The Burlington locality exhibits an unusually great differentiation of 21.7% among its two populations with 78.3% of the total genetic variation within the populations themselves.

Overall, the patterns of heterozygosity reduction seem to indicate little gene flow among the populations of Ascaris suum. The Burlington locality appears to oppose this overall trend; however, several factors must be considered in the analysis of all these data. First of all, the populations under consideration are not natural populations. The pigs are kept in confined quarters and are not free to migrate as a natural population would be, thus the Ascaris suum individuals do not migrate freely either. Second, the size of the individual populations are too small to be considered accurate statistically. RAPD markers which were scored

as always present or always absent in a given population may appear that way only because too few individuals have been tested. Finally, the inability to score heterozygotes with the RAPD technique requires investigators to use the Hardy-Weinberg formula to estimate the frequency of heterozygotes in the population. Violations of Hardy-Weinberg assumptions (e.g., natural selection) may make this estimate inaccurate.

Further research with the use of isozyme and RFLP techniques will allow for a much more complete look at the genetic variability of these populations. It may also be useful to obtain more localities, more individuals, and more localities containing more than one population to increase the accuracy of the estimate. However, the RAPD fingerprinting approach has proven to be a useful tool for obtaining data on nucleic acid polymorphisms in ascaridoid nematodes.

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Table 1: Statistics for Seven Individual Populations

	FIT	FIS	FST	HO	HS	HT
Primer 302						
1046bp	0.2549	0.0523	0.2138	0.3814	0.4025	0.5119
2337bp	0.1912	0.0573	0.1420	0.3400	0.3607	0.4204
Primer 306						
1046bp	0.2095	0.0541	0.1643	0.3857	0.4078	0.4880
1600bp	0.2096	0.0492	0.1687	0.2671	0.2810	0.3380
Primer 308						
738bp	0.1402	0.0555	0.0896	0.2514	0.2662	0.2924
Primer 314						
707bp	0.4666	0.0513	0.4378	0.1243	0.1310	0.2330
800bp	0.9128	0.0103	0.9119	0.0229	0.0231	0.2621
861bp	0.4104	0.0459	0.3820	0.2771	0.2905	0.4700
Primer 316						
584bp	0.2280	0.0444	0.1922	0.3429	0.3588	0.4441
720bp	0.0757	0.0504	0.0266	0.4186	0.4408	0.4528
1046bp	0.4439	0.0543	0.4120	0.2843	0.3006	0.5112
Primer 322						
738bp	0.1246	0.0464	0.0820	0.4486	0.4707	0.5124
Primer 337						
676bp	0.2768	0.0639	0.2275	0.3686	0.3937	0.5097
873bp	0.1991	0.0503	0.1566	0.3257	0.3430	0.4067
923bp	0.1552	0.0461	0.1144	0.4114	0.4313	0.4870
Primer 345						
984bp	0.2175	0.0714	0.1574	0.3343	0.3600	0.4272
Primer 348						
756bp	0.2512	0.0479	0.2136	0.2629	0.2761	0.3510
830bp	0.4433	0.0478	0.4153	0.2814	0.2956	0.5055

Mean FST across all markers = .2347

Table 2: Statistics for Five Locality Populations*

	FIT	FIS	FST	HO	HS	HT
Primer 302						
1046bp	0.1182	0.0383	0.0831	0.4540	0.4721	0.5149
2337bp	0.0774	0.0400	0.0390	0.3600	0.3750	0.3902
Primer 306						
1046bp	0.1800	0.0353	0.1499	0.3840	0.3981	0.4683
1600bp	0.2466	0.0407	0.2147	0.3480	0.3627	0.4619
Primer 308						
738bp	0.1054	0.0411	0.0671	0.2860	0.2983	0.3197
Primer 314						
707bp	0.4267	0.0360	0.4053	0.1000	0.1037	0.1744
800bp	0.2244	0.0396	0.1924	0.0960	0.1000	0.1238
861bp	0.2471	0.0369	0.2182	0.3580	0.3717	0.4755
Primer 316						
584bp	0.1860	0.0353	0.1563	0.3880	0.4022	0.4767
720bp	0.0579	0.0393	0.0193	0.4280	0.4455	0.4543
1046bp	0.3761	0.0417	0.3489	0.3200	0.3339	0.5129
Primer 322						
738bp	0.1115	0.0346	0.0797	0.4440	0.4599	0.4997
Primer 337						
676bp	0.3357	0.0419	0.3066	0.3400	0.3549	0.5118
873bp	0.1618	0.0384	0.1283	0.3660	0.3806	0.4367
923bp	0.0721	0.0359	0.0375	0.4680	0.4854	0.5044
Primer 345						
984bp	0.0619	0.0435	0.0193	0.3520	0.3680	0.3752
Primer 348						
756bp	0.2138	0.0349	0.1854	0.2560	0.2653	0.3256
830bp	0.2808	0.0414	0.2497	0.3660	0.3818	0.5089

* localities = Fulton, IL
Hinckley, IL
Indianapolis, IN
Burlington, MI
Cassopolis, MI

Mean FST across all markers = .1560

Table 3: Statistics for Populations from Two Cassopolis, Michigan Pigs

	FIT	FIS	FST	HO	HS	HT
Primer 302						
1046bp	0.1599	0.0627	0.1036	0.4150	0.4428	0.4940
2337bp	0.0811	0.0682	0.0138	0.2700	0.2898	0.2938
Primer 306						
1046bp	0.1949	0.0674	0.1368	0.4250	0.4557	0.5279
1600bp	0.1103	0.0603	0.0531	0.3350	0.3565	0.3765
Primer 308						
738bp	0.1150	0.0730	0.0453	0.2200	0.2373	0.2486
Primer 314						
707bp	no heterozygosity					
800bp	no heterozygosity					
861bp	no heterozygosity					
Primer 316						
584bp	no heterozygosity					
720bp	0.0838	0.0679	0.0171	0.3850	0.4130	0.4202
1046bp	0.1302	0.0606	0.0741	0.4600	0.4897	0.5289
Primer 322						
738bp	0.1018	0.0545	0.0500	0.4750	0.5024	0.5288
Primer 337						
676bp	0.0640	0.0606	0.0037	0.4600	0.4897	0.4915
873bp	0.0600	0.0596	0.0004	0.4500	0.4785	0.4787
923bp	0.0600	0.0596	0.0004	0.4500	0.4785	0.4787
Primer 345						
984bp	0.0720	0.0520	0.0211	0.4300	0.4536	0.4634
Primer 348						
756bp	0.0912	0.0651	0.0279	0.4700	0.5027	0.5172
830bp	0.2429	0.0518	0.2016	0.2300	0.2426	0.3038

Mean FST across all markers = .0519

Table 4: Statistics for Populations from Two Burlington, Michigan Pigs

	FIT	FIS	FST	HO	HS	HT
Primer 302						
1046bp	0.3550	0.0480	0.3225	0.2500	0.2626	0.3876
2337bp	0.2366	0.0569	0.1905	0.3950	0.4188	0.5174
Primer 306						
1046bp	0.0819	0.0547	0.0288	0.4450	0.4708	0.4847
1600bp	0.0240	0.0046	0.0195	0.0400	0.0402	0.0410
Primer 308						
738bp	0.1235	0.0318	0.0947	0.1500	0.1549	0.1711
Primer 314						
707bp	0.1587	0.0456	0.1185	0.4350	0.4558	0.5170
800bp	0.9202	0.0046	0.9198	0.0400	0.0402	0.5010
861bp	0.3638	0.0302	0.3440	0.2900	0.2990	0.4558
Primer 316						
584bp	0.0413	0.0364	0.0051	0.4600	0.4774	0.4798
720bp	0.0702	0.0455	0.0258	0.4050	0.4243	0.4356
1046bp	0.4650	0.0488	0.4376	0.2350	0.2471	0.4393
Primer 322						
738bp	0.0436	0.0435	0.0001	0.4700	0.4914	0.4914
Primer 337						
676bp	0.0413	0.0364	0.0051	0.4600	0.4774	0.4798
873bp	no heterozygosity					
923bp	0.3068	0.0362	0.2808	0.2500	0.2594	0.3606
Primer 345						
984bp	0.1982	0.0569	0.1499	0.1950	0.2068	0.2432
Primer 348						
756bp	0.2122	0.0353	0.1834	0.2200	0.2280	0.2792
830bp	0.3367	0.0447	0.3057	0.2500	0.2617	0.3769

Mean FST across all markers = .2171