Transactions of the Illinois Academy of Science
(1987), Volume 80, 3 and 4, pp. 309-316

THE INFLUENCE OF GENIC HETEROZYGOSITY ON THE POTENTIAL PRODUCTIVITY OF WHITE-TAILED DEER

Mark D. Maffei
and
Alan Woolf
Cooperative Wildlife Research Laboratory
Southern Illinois University
Carbondale, IL 62901

ABSTRACT

Increased genic heterozygosity has been shown to be positively correlated with phenotypic characters such as weight and antler development in white-tailed deer (*Odocoileus virginianus*). In this study, horizontal starch gel electrophoresis was used to determine heterozygosity (H) for 68 female white-tailed deer from which reproductive tracts were collected. Corpora lutea and corpora albicantia were counted in sectioned ovaries. While H was not significantly correlated to ovulation rates as measured by corpora lutea counts ($F_{3,63} = 2.04$, $p = 0.1161$), H was significantly correlated with corpora albicantia counts ($F_{3,44} = 3.09$, $p = 0.0365$). No significant differences in H were found between age classes. It appears increased heterozygosity increases potential productivity in white-tailed deer, and does with higher levels of genic heterozygosity ovulate later than those with lesser amounts of genic heterozygosity.

INTRODUCTION

The selective advantage of individuals with high levels of genetic heterozygosity has long been accepted. Two alleles at a locus can be maintained at high frequencies within a population if the fitness of heterozygotes is higher than either homozygote (Mayr 1970). This phenomenon, termed balanced polymorphism (Ford 1945), allows the maintenance of higher levels of genic variability than expected. Indirect evidence of heterozygote superiority comes from observations that severe inbreeding leads to

a reduction of fitness. "Inbreeding depression" can manifest itself in many ways, i.e. metabolic disturbances, growth anomalies, increased susceptibility to disease, and loss of fertility (Lerner 1945).

High levels of heterozygosity have been linked to increased aggressiveness and exploratory behavior in the oldfield mouse (*Peromyscus polionotus*) (Garten 1976, 1977). In dark-eyed juncos (*Junco hyemalis*), social dominance is positively correlated with heterozygosity (Baker and Fox 1978). Smith et al. (1975) found a relationship between heterozygosity and reproductive success in rodents, and predicted that reproductive, behavioral, morphological, and physiological traits of most vertebrates are positively correlated with genic heterozygosity.

In white-tailed deer, high levels of heterozygosity have been related to antler growth (Smith et al. 1983), increased fetal growth rate and doe size (Cothran et al. 1983). Adult female deer with high heterozygosity tend to produce more offspring than does with low levels of heterozygosity (Johns et al. 1977). Yearling does have been shown to be particularly affected by heterozygosity levels; those with high heterozygosity being more likely to bear twins (Cothran et al. 1983). Additionally, there appears to be a tendancy for does with high levels of heterozygosity to ovulate later than those with low levels (Baccus et al. 1977).

The effects of varying heterozygosity on the productivity of white-tailed deer have begun to be identified. To date, the majority of work on heterozygosity in deer has been limited to a semi-confined population in Georgia. There is a need to better understand this phenomenon for white-tailed deer in general. The work reported here was an effort to identify the effects of genic heterozygosity on potential productivity of a white-tailed deer herd in southern Illinois.

METHODS AND MATERIALS

Liver samples and reproductive tracts were collected from 68 female white-tailed deer harvested in late November and early December on the Crab Orchard N.W.R. during the 1980 and 1981 Illinois firearms deer hunt. Animals sampled were aged on the basis of tooth eruption and wear patterns (Severinghaus 1949). Liver samples were stored in 0.85% saline at −20°C until processed. Reproductive tracts were inspected for fetuses and preserved in 10% formalin (Haugen 1963). Ovaries were sectioned and analyzed for corpora albicantia and corpora lutea following Cheatum's (1949) technique as modified by Teer et al. (1965).

Tissue extracts were prepared for electrophoresis by mixing 0.5 g of liver with an equal volume of buffered grinding solution (0.1 M tris, 0.001 M ethylene-diaminetetraacetate (EDTA), and 5×10^{-5} M NADP, pH adjusted to 7.0 with hydrochloric acid) (Selander et al. 1971). The mixture was ground with a mortar and pestle; tissue extracts were kept on wet ice during preparation. Extract not used immediately was frozen; all were used within 48 hours of preparation.

Electrophoretic procedures followed those of Manlove et al. (1975) with modifications as needed. Three protein systems were examined; sorbitol dehydrogenase (SDH), α-glycerophosphate dehydrogenase (GPD), and esterase-2 (ES-2). GPD and SDH were examined using a discontinuous tris-citrate buffer, while ES-2 was examined using a lithium hydroxide buffer (Manlove et al. 1975, Maffei 1985).

An EC-452 500 volt power source (E-C Apparatus Corporation, St. Petersburg, Florida) provided a constant current of 75 ma through a starch gel. The gel was sup-

ported on electrode buffer chambers which contained appropriate buffer solution. Electrolyte cells, with internal dimensions of 43 mm X 50 mm X 215 mm and equipped with platinum wire electrodes, were used. Gels were prepared with potato starch hydrolyzed for electrophoresis (Sigma Chemical Co., St. Louis, Missouri). Approximately 40 g of starch were mixed with 400 ml of gel buffer, heated with agitation over an open flame until boiling, aspirated for one minute, then poured into a 196 mm X 178 mm X 9 mm gel mold. The gel, allowed to cool for 15 minutes, was covered with plastic wrap and allowed to further cool to ambient. Samples were applied to the gel by absorbing on 5 mm X 10 mm pieces of filter paper; these were then placed in a vertical slit (origin) made in the gel 60 mm from one end. Gels were arranged so they rested on buffer trays with the origin nearest the cathode. Cellulose sponge cloths served as buffer wicks by placing one end of a sponge in an electrode chamber and the other end over the terminal 30 mm of gel. Cathodal to the origin, the sponge was placed on the gel and covered with plastic wrap, while anodal to the origin, the plastic wrap was folded so that 30 mm of gel were exposed. The wick was placed over both gel and plastic wrap.

Electrophoresis was performed in a refrigerator at 4°C to prevent denaturation of proteins due to coulomb heating. On completion of electrophoresis, each gel was cut transversely into 3-mm thick slices which were stained with the appropriate solution (Maffei 1985). Scoring was accomplished by measuring to the nearest mm the distance from the origin to the leading edge of each band present; superscript values were assigned to alleles to represent the migration distance of an allele relative to the migration distance of the most common allele at that locus. Scored gels were fixed in a 5:5:1 water:methanol:acetic acid solution and wrapped for storage.

Statistical analysis were conducted using the Statistical Analysis System (SAS) package (Helwig and Council 1979). Two-way ANOVA was used to answer the following questions; 1) is knowledge of deer age and heterozygosity predictive of ovulation rate (as measured by the number of corpora lutea or corpora albicantia present)? 2) is knowledge of deer age predictive of ovulation rate over and above the effects of heterozygosity? and 3) is knowledge of heterozygosity predictive of ovulation rate over and above the effects of age? Heterozygosity was treated as a class variable with animals classed according to the number of heterozygous loci possessed. Duncan's multiple range test was used to test for between age class differences in heterozygosity.

RESULTS AND DISCUSSION

Two alleles were identified at the ES-2 and GPD loci, 3 were identified at the SDH locus (Figure 1). The frequency of the most common ES-2 allele, $ES\text{-}2^{100}$, was 0.514. A subband occurred about midway between the primary bands, and did not affect scoring (Figure 1A). The frequency of GPD^{100} was 0.713; subbanding also was observed at this locus (Figure 1B). The three alleles identified at the SDH locus, SDH^{100}, SDH^{42}, and SDH^{101}, occurred at rates of 0.654, 0.331, and 0.015, respectively. SDH is a tetramer, and heterozygotes show a pattern with 5 bands (Figure 1C).

An average of 1.223 heterozygous loci per doe was found. Fawns possessed the fewest heterozygous loci per doe, having 0.895. Does older than 4.5 years possessed an average of 1.5 heterozygous loci. These differences were not significant at the 0.05 confidence level, and are similar to results obtained for 673 white-tailed deer examined on Crab Orchard N.W.R. (Maffei 1985).

Forty-five of 49 does 1.5 years of age or older had ovulated at time of collection. Mean corpora lutea (C.L.) per doe with C.L., and mean corpora albicantia (C.A.) per doe with C.A. increased with age (Table 1). Analysis of variance indicated no significant relationship between C.L. and the number of heterozygous loci ($F_{3,69}$ = 2.04, p = 0.1161) (Figure 2). However, the occurrence of C.A. was significantly correlated to heterozygosity ($F_{3,11}$ = 3.09, p = 0.0365) (Figure 3).

Six of 18 yearling does possessed C.A. (Table 1), indicating conception as fawns. Of these 6, 4 were heterozygous at 2 loci, 1 was heterozygous at 3, and the last was heterozygous at 1 locus. Of 12 yearling does without C.A., 8 were heterozygous at 1 locus, 2 were heterozygous at 2 loci, and 2 were homozygous at 3 loci. Thus, heterozygosity may play a role in the reproductive performance of fawns on Crab Orchard N.W.R.

Heterozygosity may also influence the time of breeding, with more heterozygous does ovulating later than those with less heterozygosity (Baccus et al. 1977, Cothran et al. 1983). This may explain the lack of correlation between the number of C.L. and heterozygous loci possessed. The effect of heterozygosity on time of breeding may be due to increased productivity of young does with high heterozygosity (Cothran et al. 1983). If a fawn conceives, or a yearling doe conceives twins, energy demands will be higher than for fawns which bear no offspring, or yearlings which bear a single fawn. The resulting energy deficit may delay her next conception (Cothran et al. 1983).

Nutrition has been suggested (Kirkpatrick 1975) to be of primary importance in determing the onset of puberty in white-tailed deer fawns, and is a factor in determining ovulation rates in older deer (Nelson 1984). Nelson (1984) found that fawns which ovulated were significantly heavier than those which had not, with differences in body fat levels being particularly evident. Body weight of white-tailed deer has been shown to be significantly correlated with the number of heterozygous loci possessed (Cothran et al. 1983, Maffei 1985). It is probable that nutrition is of primary importance in determining onset of puberty and ovulation rates in white-tailed deer, while heterozygosity plays a role in determining ovulation rates within bounds set by the general health and nutritional state of an animal.

ACKNOWLEDGEMENTS

We thank the staff of Crab Orchard N.W.R. for access to the study area, and the hunters that provided samples. Funding was provided through the Illinois Department of Conservation Federal Aid to Wildlife Restoration Project W-63-R(SI), and the Cooperative Wildlife Research Laboratory, Southern Illinois University at Carbondale.

LITERATURE CITED

Baccus, R., H.O. Hillestad, P.E. Johns, M.N. Manlove, R.L. Marchinton, and M.N. Smith. 1977. Prenatal selection in white-tailed deer. Proc. Annual Conf. S.E. Assoc. Fish and Wildl. Agencies 31:173-179.

Baker, M.C., and S.F. Fox. 1978. Dominance, survival and enzyme polymorphism in dark-eyed juncos, *Junco hyemalis*. Evolution 32:697-711.

Cheatum, E. 1949. The use of corpora lutea for determining ovulation incidence and variations in fertility of white-tailed deer. Cornell Vet. 39:282-291.

Cothran, G.C., R.K. Chesser, M.H. Smith, and P.E. Johns. 1983. Influences of genetic variability and maternal factors of fetal growth in white-tailed deer. Evoluation 37:282-291.

Ford, E.B. 1945. Polymorphism. Biol. Rev. 20:73-88.

Garten, C.T., Jr. 1976. Relationship between aggressive behavior and genic heterozygosity in the old-field mouse, *Peromyscus polionotus*. Evolution 30:59-72.
Garten, C.T., Jr. 1977. Relationship between aggressive behavior and genic heterozygosity in the old-field mouse. Anim. Behav. 25:328-332.
Haugen, A. 1963. An improved technique for preserving deer uteri. J. Wildl. Manage. 27:142-143.
Helwig, J.T., and K.A. Council. 1979. SAS user's guide: 1979 edition. SAS Institute Inc., Raleigh, North Carolina. 494 pp.
Johns, P.E., R. Baccus, M.N. Manlove, J.E. Pinder, and M.H. Smith. 1977. Reproduction patterns, productivity, and genetic variability in adjacent white-tailed deer populations. Proc. Annual Conf. S.E. Assoc. Fish and Wildl. Agencies 31:167-172.
Kirkpatrick, R.L. 1975. Nutrition, hormones, and reproduction in white-tailed deer: a review. Proc. N.E. Deer Study Group 1:181-193.
Lerner, I.M. 1945. Genetic homeostasis. John Wiley, New York. 134 pp.
Maffei, M.D. 1985. Spatial heterogeneity of allele frequencies in white-tailed deer on Crab Orchard National Wildlife Refuge. Ph.D. Dissertation. Southern Illinois University, Carbondale. 149 pp.
Manlove, M.N., J.C. Avise, H.O. Hillestad, P.R. Ramsey, M.H. Smith, and D.O. Straney. 1975. Starch gel electrophoresis for the study of population genetics in white-tailed deer. Proc. Ann. Conf. S.E. Assoc. Game and Fish Commrs. 29:392-403.
Mayr, E. 1970. Populations, species, and evolution. Harvard Press, Cambridge, Massachusetts. 453 pp.
Nelson, T.A. 1984. The production and survival of white-tailed deer fawns on Carb Orchard National Wildlife Refuge. Ph.D. Dissertation, Southern Illinois University, Carbondale, 1964 pp.
Selander, R.K., M.H. Smith, Y. Yang, W.E. Johnson, and J.B. Gentry. 1971. Biochemical polymorphism and systematics of the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. University of Texas Publ. 7103:49-90.
Severinghaus, C.W. 1949. Tooth development and wear as criteria of age in white-tailed deer. J. Wildl. Manage. 13:195-216.
Smith, M.H., R.K. Chesser, E.G. Cothran, and P.E. Johns. 1983. Genetic variability and antler growth in a natural population of white-tailed deer. Pages 365-388 *in* R.D. Brown (ed.), Antler development in cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, Texas.
Smith, M.H., C.T. Garten, and P.R. Ramsey. 1975. Genic heterozygosity and population dynamics in small mammals. Pages 385-402 *in* C.L. Markert (ed.), Isozymes IV: genetics and evolution. Academic Press, New York.
Teer, J., J. Thomas, and E. Walker. 1965. Ecology and management of white-tailed deer in the Llano basin of Texas. Wildl. Monogr. 15:1-62.

Table 1. Occurrences of corpora lutea (C.L.) and corpora albicantia (C.A.) in white-tailed deer does harvested on Crab Orchard N.W.R., 1980 and 1981.

Age[A]	N	No. with C.L.	No. with C.A.	ΣC.L.	ΣC.A.	XC.L.[B]	XC.A.[C]
0.5	19	3(16%)	0	4	0	1.33	
1.5	18	17(94%)	6(33%)	30	7	1.76	1.17
2.5	11	9(82%)	11(100%)	17	17	1.89	1.54
3.5	6	6(100%)	6(100%)	10	10	1.67	1.67
4.5	6	6(100%)	6(100%)	16	13	2.67	2.33
4.5+	8	7(88%)	8(100%)	17	20	2.43	2.50

[A]Aging based on tooth wear.
[B]X corpora lutea per doe with corpora lutea.
[C]X corpora albicantia per doe with corpora albicantia.

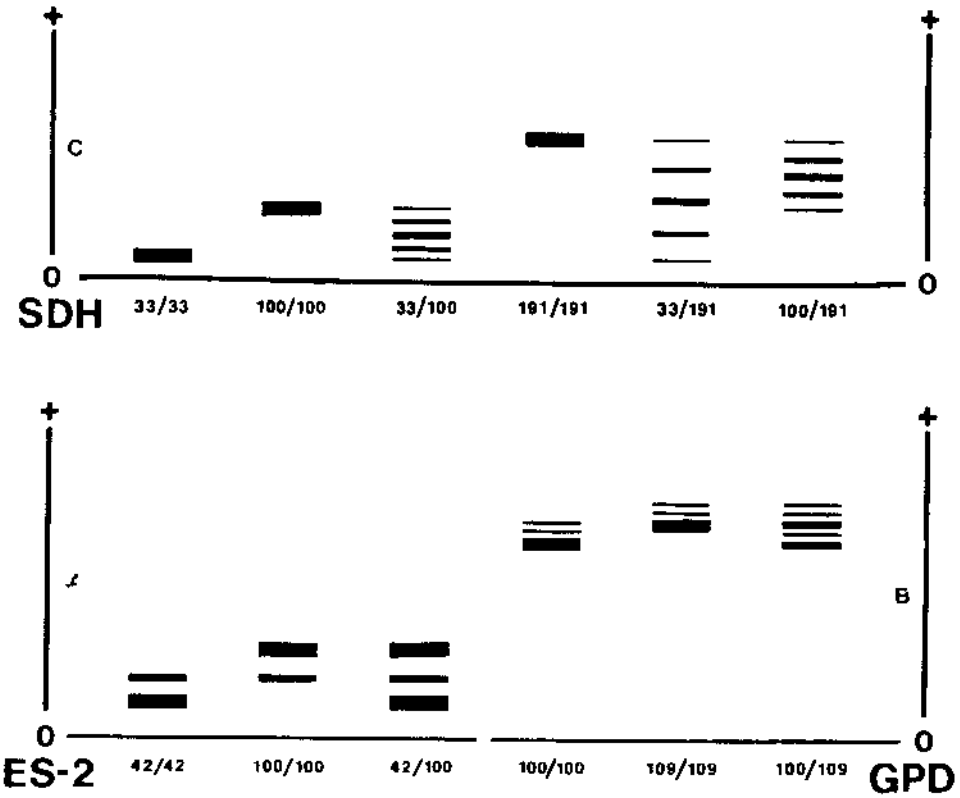

Fig. 1. Banding patterns of 3 polymorphic proteins in white-tailed deer. Allelic designations are given below the origin (0-0). (A) Esterase-2; (B) α-glycerophosphate dehydrogenase; (C) Sorbitol dehydrogenase. Banding patterns were not always as distinct as illustrated.

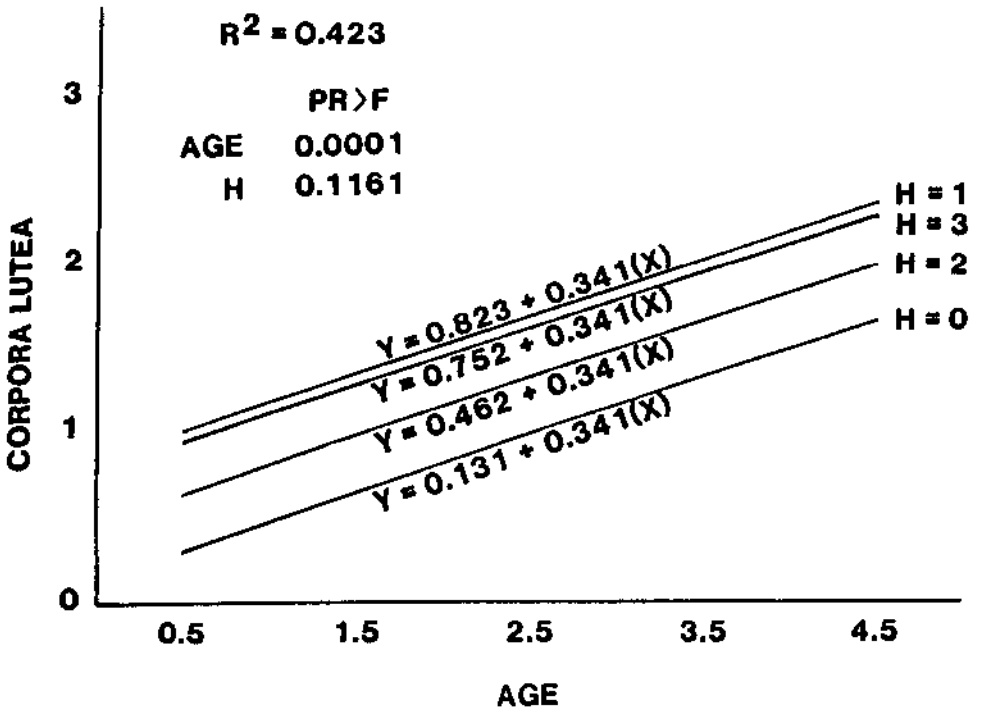

Fig. 2. Relationship between age and number of heterozygous loci (H) in female white-tailed deer and occurrence of corpora lutea. H is represented as the number of heterozygous loci possessed by an individual. Figure illustrates results for 68 animals.

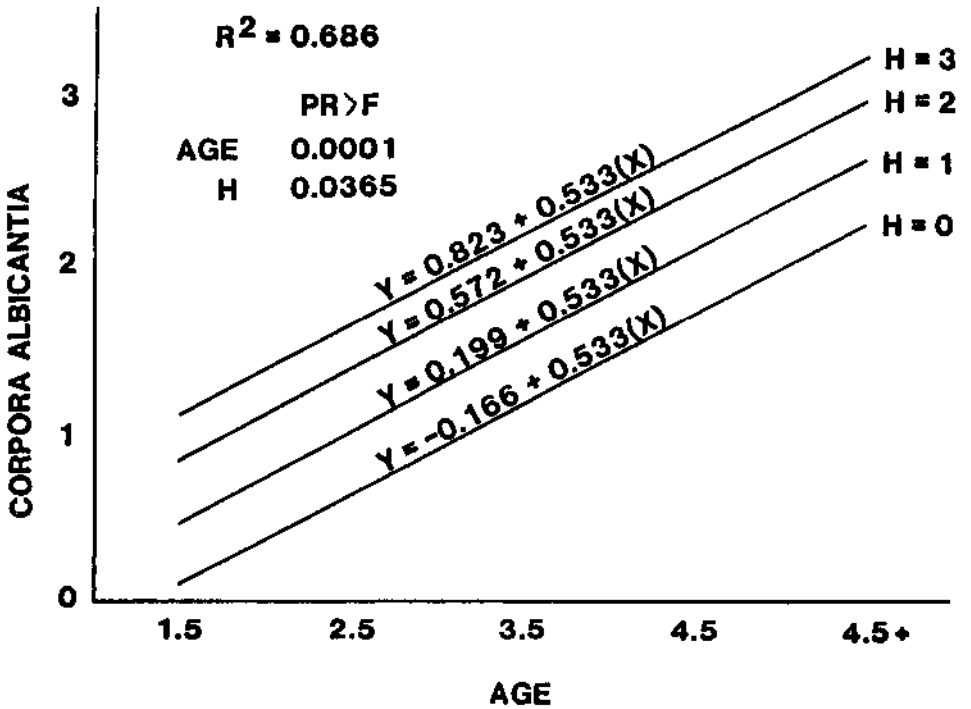

Fig. 3. Relationship between age and number of heterozygous loci (H) in female white-tailed deer and occurrence of corpora albicantia. H is represented as the number of heterozygous loci possessed by an individual. Figure illustrates results for 49 animals.