The inheritance of behavioral wildness in house mice (*Mus musculus L.*)

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Fourteen behavioral measures were ranked according to their ability to differentiate performances of a wild population of mice from three domestic inbred strains. These "wildness" rankings were then analyzed for their ability to predict genetic and Genotype by Environment interactions. Genetic parameters were derived from analysis of three inbred domestic strains and their three hybrid crosses via the diallel technique of genetic analysis. Genotype by Environment interactions were assessed both as mean differences in stock performances resulting from rearing in a laboratory vs naturalistic environment and as differences in environmental variability within stock genotypes. Behavioral characters ranking high on "wildness" failed to demonstrate any unitary pattern of Genotype by Environment interactions. The diallel genetic analysis revealed many examples of additive variation but relatively few examples of dominant variation. An extremely wide range of interactions occurred between genotype and rearing environments, with "wildness" and "developmental homeostasis" concepts both failing to account for all the observed interactions.

Since the origin of behavioral psychology as an independent discipline, many studies have attempted to define "wildness" and determine the nature of its inheritance (Yerkes, 1913; Coburn, 1922; Dawson, 1932, Stone 1932; Keeler, 1942; Galef, 1970). Nearly all of these studies on rodents make at least one of two largely untested assumptions.

First, they assume that the behaviors which distinguish wild rodents from domestics are closely interrelated and may be organized into traits. This assumption is either explicit (Galef, 1970) or implicit in the construction of rating scales which lump together scores from several distinct types of behavior into a single summary statistic (Yerkes, 1913; Coburn, 1922; Stone, 1932). For example, on the basis of descriptive similarities Yerkes grouped (a) biting, (b) teeth gnashing, (c) jumping, and (d) vocalization into the trait of rat "savageness"; similarly, Yerkes grouped (e) avoidance, (f) cowering, (g) urination, and (h) defecation into the trait of rat "timidity." A common "savageness" or "timidity" score was derived by rating the presence/absence of each component behavior (biting, gnashing, etc.) and summing these ratings into a single score. This method of scoring is valid only if the presence of one component behavior, such as biting, is correlated with the presence of the other components, such as gnashing.

Second, nearly all previous studies assumed that the behaviors characterizing wild rodents were "simply"

inherited, i.e., were controlled by a small number of genes (Coburn, 1922; Dawson, 1932; Keeler, 1942). This latter assumption is most dubious. Modern genetic analyses indicate that behavioral characters, which usually vary quantitatively and are far from the source of primary gene action, are typically inherited by polygenetic mechanisms (Hall, 1951; Caspari, 1958).

The present study tested the assumption that "wildness" was a unitary trait and applied modern quantitative-genetic methods to the analysis of several behaviors highly distinctive of wild mice. A population of wild mice was compared with six stocks of domestic inbreds and domestic hybrids on a test battery of 15 behavioral characters (Smith, 1972). These seven genetic stocks were raised in two different habitat environments: a simulated naturalistic environment and a laboratory environment. The behavioral comparisons allow an objective assessment of a character's ability to measure "wildness." All 15 characters were ranked on their overall ability in consistently differentiating the performance of the wilds from that of the domestics. If wildness is a unitary trait, then characters with a high rank on "wildness" should show similar patterns of inheritance and reactivity to manipulation of habitat environment.

In the field of quantitative genetics, techniques of analysis have been developed that can be applied to polygenetically inherited behavioral characters. The diallel cross method of genetic analysis, using all possible crosses among inbred (homozygous) strains, is one such method. Since tests were run on three domestic inbred strains and their three hybrid crosses, this design permitted the application of the diallel technique.

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METHOD

Subjects

Ss were 204 mice (*Mus musculus* L.), 36 from a laboratory maintained population of wild mice, 36 each from three inbred strains (C57/BL6, DBA/2, A/J), and 20 each from their hybrid crosses (C57 by DBA, C57 by A/J, DBA by A/J).¹ The wild mice were third and fourth generation animals derived from 16 original breeding pairs of feral *Mus musculus* live-trapped in a factory. All generations of wild mice were randomly mated in a manner which doubled the number of effective breeding pairs by restricting variations in family size (Falconer, 1962). The number of effective breeding pairs was 24 per generation. Half of the Ss were male and half were female. Animals were 90-120 days old at testing, and age distributions were comparable within stocks. All animals descended from breeders maintained by Hugh Bradford at the University of Pennsylvania.

Apparatus

Half of the Ss were raised in a standard laboratory environment patterned after that of The Jackson Laboratory. Animals in the laboratory environment were housed in 8 x 11 x 6 in. plastic cages covered by wire tops. Litters were kept together with their mother until weaning, at which time the mother was removed. The colony room was separated from other activity and was disturbed only for routine animal maintenance. The remaining half was raised in a simulated naturalistic environment. The naturalistic environment was a wire-mesh cage, 4.5 x 3 x 1.5 ft. Within the cage additional climbing areas were provided by a wire-mesh jungle gym and a Masonite balcony floor. Small tin cans, Sanicel, and paper toweling provided cover on the floor areas. Water bottles were fastened to the outside of the cage, and converted pigeon feeders provided food. The pigeon feeders served additionally as devices for capturing test animals.²

The following devices were employed for behavioral testing: (1) Activity testing employed a 9-in.-diam closed running wheel which was connected to a remote recorder measuring revolutions. (2) Two-way signalized avoidance testing employed a Lehigh Valley shuttlebox (No. 146-02). The CS consisted of a 5-sec buzzing noise presented together with a flickering light; the US consisted of a .02-mA scrambled footshock which was maintained until the S shuttled to the other side of the box (escape). If the S shuttled during the CS presentation, however, the CS was terminated and shock delivery was aborted (avoidance). (3) Emergence testing utilized a 4 x 16 x 9 in. alleyway attached to a home cage, 8 x 8 x 9 in. The black home cage was darkened and its floor was covered with coarse-textured Sanicel bedding; the alleyway was illuminated and its floor painted with smooth white enamel. (4) Open-field tests were made in a galvanized tub 22 in. deep with a Masonite floor of 19-in. diam. The floor was marked with two concentric rings. The outer ring was transversely by three diameters, thus resulting in six outer and seven inner sections. Illumination was provided to the E by two red frosted 100-W bulbs suspended 24 in. above the open-field floor. The purpose of the lighting procedure was to minimize effective illumination for the Ss, while still providing sufficient light for the E to detect all the response components typical of open-field behavior. Since mice are relatively insensitive to red illumination and have poor visual ability, the dim red illumination used should have had the intended effect. (5) Underwater escape was run in a submerged runway 48 x 4 x 8 in. A wire-mesh screen .5 in. below the water formed the runway's ceiling. A 3.5 x 3 x 4 in. startbox with a guillotine door submerged the S. At the other end of the runway, a mesh ramp led to a 3 x 4 x 6 in. goalbox which allowed the S to emerge for air through a hole in the wire-mesh ceiling.

Procedure for Animal Testing

Half of the Ss were reared postnatally in the naturalistic environment, while the other half were reared in the laboratory environment. All Ss were raised in the laboratory environment until weaning age (30 days), whereupon half of all split litters were placed in the naturalistic environment, while the other half remained in the laboratory environment. Ss were reared with same-sex littermates. The average number of animals per cage in the laboratory environment was five and the average number per naturalistic environment was seven. At 90 days of age, test Ss were removed from their habitat environments and individually housed in wire rack cages for 4-6 days. After this adaptation period, animals were removed from their wire rack cages and run through a randomized series of tests: (1) Wheel activity was measured as the number of revolutions generated per day on five daily 60-min sessions. At the end of each daily session, the S was replaced in its wire rack cage. (2) Avoidance learning was measured as the percentage of CS-US presentations on which the S successfully avoided shock USs. Animals were tested for a total of five daily testing sessions. Each session consisted of 53 CS-US presentations (trials) separated by a variable intertrial interval. (3) Emergence timidity was measured as latency in seconds to emerge into the alley from home cage nesting boxes. In this test, animals were removed from their wire rack cages and placed into home cage nesting boxes for a 4-day adaptation period. An "emergence" consisted of S's placing all four paws in the alleyway. After emerging, Ss were allowed a 1-h period of free "exploratory" access to the alleyway. The maximum allowed latency for emergence was 2400 sec; Ss were tested for 5 successive days, after which they were returned to their wire rack quarters. (4) Open-field behaviors were measured as total instances over five daily sessions of freezing, rearing, jumping, grooming, urination, defecation, entry of an inner floor section, and entry of an outer section. Each daily session consisted of the S's being placed in an outer sector and being observed for behaviors displayed during the subsequent 5 min. At the end of each daily 5-min trial, the S was replaced in its wire rack cage. (5) Underwater escape was measured as emergence latencies during sessions of four trials each, run on 6 successive days. Sessions were run with a 25-min separation between individual trials. Escape latencies were measured as the interval between individual trials. Escape latencies were measured as the interval between the time when the startbox opened and the time the S broke water at the goalbox. At the conclusion of each trial, the S was replaced in his wire rack cage.

Procedure for Statistical Analysis

The data generated during testing were analyzed via three different methods: (a) First, the differences among the six domesticated strains (C57, DBA, A/J, CXD, CXA, DXA) were analyzed for genetic parameters by a diallel analysis of variance scheme developed by Hayman (1954);³ (b) second, the interaction of environment (natural vs laboratory habitat) with stock genotype was analyzed by a series of t comparisons; (c) third, all behavioral measures were ranked on their ability to measure "wildness," i.e., to separate the mean performance of the wild stock from that of the domestic stocks. Correlational procedures were then used to test the ability of a measure's "wildness" ranking to predict genetic effects (as analyzed by the preceding Methods a and b). The following discussion details the mechanics of applying these statistical methods to the experimental data.

Method of Assessing Genetic Parameters

The diallel analysis computed three genetic parameters as estimated in Hayman's model (Hayman, 1954): a_1 represents additive genetic variation (genetic variation arising from one gene allele, c_1 say, being replaced by its allelomorph, c_2 say, at the

same chromosomal locus, $c1/c2$). This is the type of genetic variation affected by artificial selection procedures. b_2 represents dominant genetic variation (genetic variation arising from one gene allele, $c1$ say, masking the effect of another gene allele, $c2$ say, when the chromosomal locus is in a heterozygous condition, $c1/c2$). This is the type of genetic variation affected by procedures which cross inbred lines to produce hybrid progeny.

These parameters were computed for all behavioral tests whose data could meet the assumptions of the diallel analysis. The diallel model assumes that the phenotypic effects of gene alleles do not interact. In the present case, that is equivalent to assuming that the parameters a_1 and b_1 are independent statistically. This "absence of genetic interaction" (termed *epistasis* in genetics) assumption was tested by both a t test and an F test (Hayman, 1954). Connor and Winston (1972) describe the mechanics of applying these tests in a diallel design identical to the present one.

Thus, the genetic parameters a_1 and b_1 were estimated for all parameters not showing significant genetic interactions (epistasis). The significance of the parameters was F tested, with the "error" variance term derived from differences in parameter estimates between the two environmental blocks. (In the present experiment, the "environmental blocks" were the naturalistic vs laboratory environments.) Thus, the "error" term consisted of a Genetic Parameters by Environmental Blocks interaction and possessed the advantage that a "general" absence of Genotype (strain) by Environment interaction need not be assumed. In practice, the error variances for each parameter were pooled after homogeneity of variance was assured by failure of Bartlett's test. Hence, the statistical significance of the genetic parameters a_1 and b_1 indicated the presence of additive and dominant genetic variation for the behavioral measures. Connor and Winston (1972) describe the computational details for significance testing of these genetic parameters.

In addition to the diallel parameters, an assessment was made of heterosis in the behavioral measures. A measure exhibits heterosis, or "hybrid vigor," if the mean performance of hybrid progeny consistently falls outside the range of their inbred parent means. An example would be hybrid strains whose wheel activity (number of revolutions) exceeded the activity levels of their most active inbred parents. The presence of heterosis was assessed by comparing the overall mean of the three hybrid strains (CXD, CXA, DXA) with this contrast: twice the mean of the "best" (nearest scoring) inbred parent plus once the mean of the "next best" parent, all divided by 3. A significant t difference results only if hybrid performance consistently fell outside the range of inbred performance. Theoretically, most cases of heterosis are due to the masking of "deleterious" (performance reducing) recessive genes in hybrids that are unmasked in their inbred parents. Hence, characters exhibiting significant heterosis should also exhibit dominant modes of inheritance (Falconer, 1962).

Method of Assessing Genotype by Environment Interactions

Genotype by Environment interactions were of two differing forms,

(I) The rearing of Ss in a laboratory vs naturalistic postnatal environment produces different effects in different genetic stocks. For example, laboratory rearing could enhance performances in the wild stock but have no effect on or depress performances in domestic stocks. This result would occur if the genetic consequences of domestication had altered the reactivity of laboratory strains to environmental manipulation. Alternatively, laboratory rearing could enhance or depress performance in domestic inbred strains but exert no effect upon performance of domestic hybrids. Such a result would occur if heterozygous (c_1/c_2 , d_1/d_2 , etc.) genotypes rendered hybrids more resistant to environmentally induced alterations produced during the animal's development ("developmental homeostasis"). The presence of these Genotype by Postnatal

Environment interactions were tested by t tests for the effects of habitat environment in the following stocks: wilds, domestic inbreds (A/J, C57, DBA), and domestic hybrids (AXC, AXD, CXD). The effects of domestication and developmental homeostasis would be detectable as differing habitat effects among the different stock genotypes.

II. A second type of Genotype by Environment interaction results from the "minor" environmental differences within each habitat environment. Each of the six domestic strains represent a collection of genetically identical animals. Hence, any variation in performance within these stocks must be due to environmental differences. One source of environmental differences results from the just discussed habitat differences. However, additional environmentally induced differences arise within each of the 12 domestic genotype/habitat groups. These within-group error variances can interact with genotype in ways similar to the previously discussed variances produced by habitat differences. That is, if domestic hybrids are more resistant to environmental alteration than inbreds (developmental homeostasis), then hybrid strains should manifest smaller amounts of within-habitat "error" variance. This possibility was tested by estimating coefficients of variation (error variance mean) for hybrid vs inbred stocks. The dividing of the strain error variance by the strain mean assures that a larger variance is not a simple by-product of a larger average performance. Hence, "developmental homeostasis" would be reflected in a consistently smaller coefficient of variation for hybrid vs inbred strains.

Method of Assessing Relationships Between Wildness and Genetic Effects

A third form of statistical analysis concerned itself with the ability of "wildness" to predict genetic parameters and Genotype by Environment interactions. The general question asked is, "Do behavioral measures which best differentiate wild from domestic populations possess a common mode of inheritance and a common pattern of reactivity to environmental manipulation?" In order to answer this question, the behavioral characters must first be ranked according to their ability to "measure wildness," i.e., differentiate the performance of wilds from domestics. A character which "measures wildness" well should possess the following properties: (a) There should be a large absolute difference between the typical performances of wilds and domestics, and (b) this difference should be consistent across individuals. Hence, a statistic was generated which would be large if the absolute differences between wilds and domestics were large, replicable, and consistent. The statistic was an F contrast between the wild stock and all of the three domestic inbred strains (C57, DBA, A/J). In this statistic, the msq treatment of the contrast was divided by the overall msq error. A large overall pattern of differences between the mean of wilds and all inbred domestics would produce a large msq treatment. However, this msq treatment would be reduced if stocks differed by other patterns or if the overall wilds vs domestic difference interacted with habitat conditions. The size of the ratio would also be reduced by large individual differences within stocks (errors). Hence, this differentiability parameter of "wildness" would be large only if the wild vs domestic differences were themselves large, replicable, and consistent.

Hence, if "wildness" is a unitary trait, then those behaviors exhibiting high scores on the differentiability parameter should also exhibit similarities in estimates of inheritance and Genotype by Environment interactions. Although it is easy to determine the presence or absence of such a correlation by cursory examination of the data, statistical testing of its magnitude is impeded by dependencies among behavioral measures. That is, while the order of testing was randomized, every mouse in the present study was scored on all 15 behavioral measures. Statistical dependencies between measures may arise from performance correlations both between different tests and within the same test. An example of the former would be a reduction in open-field activity resulting from previous

Table 1
Summary Statistics for Stock Performance on Behavioral Measures

Behavioral Measures Ranked According to Ability in Differentiating Wild vs Domestic Populations																
Genetic Stocks		OF Jumping	OF Freezing	Percent Avoid Day 5	Wheel	OF Urinate	Escape 6 Days	OF Defecate	OF Rear	OF Activity	OF Groom	OF W-Seek	Escape 5 Days	Escape Day 1	Percent Avoid Day 1	Escape Day 1
Data from Wild Stock																
B-W (N = 36)	M	6.73	2.28	59.94	15.98	.34	3.96	.79	.98	3.28	8.06	6.74	3.88	6.27	19.44	8.82
	SD	4.7	1.95	29.06	6.6	.29	1.04	.53	.36	1.08	5.0	1.32	4.25	2.13	16.40	9.62
Data from Inbred Domestic Stocks																
C57/6 (N = 36)	M	.44	.22	87.36	12.76	.64	4.54	.43	1.76	5.82	12.08	4.32	1.70	8.50	31.61	7.46
	SD	.63	.48	10.81	2.11	.80	1.15	.38	.53	1.03	6.33	1.1	1.48	3.9	17.8	6.93
DBA/2 (N = 36)	M	.25	.12	88.52	12.12	1.40	7.15	.91	1.54	5.31	6.58	7.08	1.52	10.02	23.11	6.71
	SD	.35	.30	9.3	2.6	1.56	2.08	.55	.38	1.30	3.33	1.60	1.78	3.9	15.5	6.5
A/J (N = 36)	M	.01	.01	82.47	9.61	.60	3.76	2.22	.07	1.37	.92	9.83	4.56	4.65	14.72	13.62
	SD	.03	.03	15.0	1.3	.35	1.10	.55	.13	.75	1.1	.22	4.1	.63	14.9	9.38
Data from Hybrid Domestic Stocks																
C by D (N = 20)	M	.83	.17	89.03	12.05	.84	3.90	.76	1.68	5.04	5.70	6.05	2.48	6.36	31.56	10.30
	SD	.79	.25	5.1	2.6	.78	.55	.39	.28	.82	3.98	.95	2.65	1.93	15.1	8.33
C by A (N = 20)	M	.60	.05	93.35	13.85	.75	3.44	1.13	1.71	4.31	3.0	6.17	1.37	5.42	29.60	10.09
	SD	.73	.08	5.8	1.55	.98	.47	.80	.3	.70	2.58	.93	1.0	1.28	18.0	7.43
D by A (N = 20)	M	.15	.10	84.91	14.30	1.50	3.89	1.47	1.27	3.58	4.20	8.55	1.54	6.41	16.54	10.30
	SD	.24	.23	16.2	2.63	.90	1.10	.75	.45	.90	3.23	.91	1.68	1.48	13.5	9.7

Note: All data in this table were summarized from Table 1 of Smith (1972). Refer to that table for analyses of environmental habitat effects.

experience in the shock avoidance task; an example of the latter would be a reduction in open-field activity due to competing open-field freezing tendencies. Hence, the following solutions were applied to the problem: (a) First, a nonparametric test, the Wilcoxon signed-rank test, was applied to the data. This test does not assume independence between experimental units (measures). Rather, it assumes that the differences in rankings are independent among the scores being correlated. In the present experiment, this is equivalent to assuming that differences are independent for rankings of stock performance on the "wildness" parameter vs the genetic parameters. Since stock performances are the ultimate source of both sets of parameters, dependencies should result only if stock performances were themselves correlated. (b) Thus, a second step was to run a correlation matrix on stock (genotype) correlations for all 15 measures. Correlated measures ($p < .05$) were then eliminated from this matrix, and the Wilcoxon rank test was performed only upon the remaining measures. Thus, this test assessed whether a measure's rank on the wildness parameter predicted rank on the genetic parameters.

RESULTS

Genetic Parameters

Generally, the behavioral characters exhibited many instances of additive variance, especially among

open-field behaviors, while exhibiting relatively few instances of dominant variance and heterosis. Data in the second row of Table 2 (Parameter B) demonstrate significant additivity for all open-field behaviors except jumping: open-field freezing, defecation, activity, urination, rearing, grooming, and wall seeking. The only other behavior exhibiting significant amounts of additive variation is underwater escape performance summed over all testing sessions.

In contrast to the additivity results, data in the third row of Table 2 (Parameter C) demonstrate significant dominance variance only for underwater escape (summed totals) and wheel activity (summed totals); no significant dominance variance was exhibited for any of the open-field behaviors. Moreover, further analysis of the wheel activity and underwater escape data revealed that genetic dominance (Parameter C) in these two measures partially resulted from certain parental inbred lines contributing more to dominance variation than others. Hybrid progeny from crosses involving A/J inbred parents (A by C, A by D) exhibited the most heterosis in wheel activity, while the A/J performance itself on this measure was the poorest (C, D > A) of any

Table 2
Wildness as a Predictor of Genetic Parameters

Parameter	Behavioral Measures Ranked According to Ability in Differentiating Wild vs Domestic Populations														
	OF Jumping	OF Freezing	Percent Avoid Day 5	Wheel	OF Urinate	Escape 6 Days	OF Defecate	OF Rear	OF Activity	OF Groom	OF W-Seek	Escape 5 Days	Escape Day 1	Percent Avoid Day 1	Escape Day 1
Differentiability Parameter: Difference in Mean Performance of Wilds vs Mean Performance of Three Domesticated Inbred Strains (Smith, 1972)															
A: Ability to differentiate wild vs domesticated (F, df = 1/123)	705.1†	377.3†	180.5†	124.4†	61.2†	58.7†	57.1†	29.0†	26.9†	20.5†	14.7†	9.5†	8.1**	5.7*	.2
Genetic Parameters: Results from Diallel Analysis of Additive, Dominant, and Heterotic Inheritance															
B: Extent of additive inheritance (F, df = 1/5)	3.1	11.7*	—	.5	7.8*	36.5†	21.6†	7.5*	18.8†	9.7*	66.4†	.5	4.4	2.3	1.4
C: Extent of dominant inheritance (F, df = 1/5)	3.0	.6	—	12.8*	1.1	50.5†	.2	3.3	.2	2.8	.7	1.6	4.4	.2	.4
D: Extent of heterosis; hybrid vs best inbred (t, df = 166)	.57	.10	.43	1.30	.19	1.21	2.86†	.89	5.07**	.50	3.01†	1.21	.27	1.44	.43

Note: Genetic parameters for additivity (B) and dominance (C) were not computed for Day 5 avoidance. Statistical tests for epistatic genetic variance revealed significance, thus violating the "no epistasis" assumption of the diallel analysis. * $p < .05$ ** $p < .01$ † $p < .005$

inbred parent (diallel analysis for b_2 parameter: $F = 8.65$, $df = 115$, $p < .025$). Similarly, hybrid progeny from crosses involving C57 inbred parents (C by D, C by A) exhibited the most heterosis in the underwater escape task, while the C57s themselves exhibited mediocre ($A > C > D$) underwater escape performance (diallel analysis for b_2 parameter: $F = 7.10$, $df = 1/5$, $p < .05$; refer to Table 1). These latter findings suggest that effects of dominance variation may be of limited generalization when extrapolated to inbred stocks different from those presently used.

Only three instances occurred whereby hybrid performance significantly exceeded the performance of their closest scoring parents (heterosis). Referring to the bottom row of Table 2 (Parameter D), the data indicate that hybrids show significantly greater open-field defecation, activity, and wall-seeking tendencies. Neither of the two measures exhibiting significant dominance variance, wheel activity and underwater escape, showed statistically significant amounts of heterosis. In part, this may be due to the severity of the heterosis criterion (hybrids must exceed best parents) compared to the dominance criterion (all hybrids must exceed all inbreds). While many of the differences between individual hybrid stocks and individual domestic stocks were statistically significant, the magnitudes of these differences were small compared to the magnitude of difference between all domestics and wilds.

Genotype by Environment Interactions

Generally, the 15 behavioral characters exhibited many Genotype by Environment interactions when the source of environmental variance was the difference between laboratory and natural habitats. However, the behavioral measures exhibited no Genotype by Environment interactions when the source of environmental variance was individual differences within strain and treatment conditions (environmental "error" variance). Data in the first three rows (E, F, G) of Table 3 reveal that laboratory rearing significantly

affected all of the measures tested except last day avoidance. None of these 14 "positive" instances, however, revealed a pattern of habitat effect which was consistent across genotypes: (a) Laboratory habitat rearing significantly depressed performance in inbreds but not hybrids or wilds on seven measures (open-field jumping, defecation, activity, grooming, underwater escape—summed, emergence—summed, and first day avoidance); (b) laboratory habitat rearing depressed performance in hybrids but not inbreds or wilds on two measures (home cage emergence on first day and open-field wall seeking); (c) laboratory habitat rearing depressed performance in wilds but not inbreds or hybrids on two measures (open-field freezing and rearing); (d) laboratory rearing enhanced performance in hybrids but not wilds on one measure (wheel activity); (e) laboratory rearing enhanced performance in wilds and inbreds but not hybrids on one measure (underwater escape, Day 1); (f) laboratory rearing enhanced performance in wilds and hybrids but depressed inbred performance on one measure (open-field activity). Thus, of the 14 "positive" instances of significant environmental effects, no instance occurs in which laboratory habitat either consistently enhances or consistently depresses performance in all genetic stocks.

Heterozygosity fails to account for all instances of interactions between genotype and environmental habitat exhibited by domestics. The only genetic difference between domestic inbreds and hybrids is the homozygosity of inbreds vs the heterozygosity of the hybrids.¹ If all Genotype by Environment interactions resulted from the superior ability of hybrids to resist environmentally induced phenotypic alterations (developmental homeostasis), then all interactions would follow a single pattern: Postnatal rearing in laboratory environments should alter (either depress or enhance) performance in "unstable" inbreds but have little or no effect on "stable" hybrids. The more heterozygous wilds should be "stable" and unaffected. While 5 of 14 Genotype by Environment interactions in Table 2 follow

Table 3
Wildness as a Predictor of Genotype by Environment Interactions

Parameter	Behavioral Measures Ranked According to Ability in Differentiating Wild vs Domestic Populations														
	OF Jumping	OF Freezing	Percent Avoid Day 5	Wheel	OF Urinate	Escape 6 Days	OF Defecate	OF Rear	OF Activity	OF Groom	OF W-Seek	Emergence 5 Days	Escape Day 1	Percent Avoid Day 1	Emergence Day 1
Interaction of Genotype with Postnatal Habitat: Effect of Being Reared in a Laboratory vs a "Natural" Environment as a Function of Stock Genotype															
E Domestic inbreds (t, df = 59)	↓***	—	—	↑*	—	↓*	↓***	—	↓***	↓**	—	↓*	↑***	↓***	—
F Domestic hybrids (t, df = 59)	—	—	—	↑*	↑*	—	—	—	↑*	↑**	↓**	—	—	—	↓*
G: Wilds (t, df = 35)	—	↓*	—	—	↑*	—	—	↓**	↑*	—	—	—	↑*	—	—
Interaction of Genotype with the Extent of Environmental Variance within Stocks: Coefficient of Variation for Within-Strain Differences as a Function of Stock Genotype															
H: Domestic inbreds; rel amt of env variance ($S^2 e/\bar{X}$ strain)	1.48	2.25	.40	.18	.28	.41	.25	.87	.30	.36	.94	.55	.14	.69	.82
I: Domestic hybrids; rel amt of env variance ($S^2 e/\bar{X}$ strain)	1.16	2.00	.10	.17	.19	.58	.19	.86	.22	.26	.99	.76	.14	.60	.83

↑ indicates that laboratory rearing increased behavioral tendencies.
— indicates no statistically significant effect.

↓ indicates that laboratory rearing decreased behavioral tendencies.
** $p < .01$ *** $p < .005$

this pattern (open-field jumping, defecation, underwater escape—totals, home cage emergence—totals, and first day avoidance), the other 9 do not. Of these nine, there are three instances of “reverse developmental homeostasis”—habitat affecting hybrid performance but not inbreds—(open-field urination, open-field wall seeking, and home cage emergence, Day 1). In the other two instances, habitat affected hybrids, but in an opposite direction to inbreds (open-field activity and grooming). In the remaining three interactions, one case occurred where habitat affected both inbreds and hybrids similarly but wilds differently (wheel activity), and two cases occurred where habitat affected scores of wilds but not those of hybrids or domestics (open-field freezing and rearing).

Statistical testing also failed to demonstrate that “developmental homeostasis” produced the differences in response between hybrids and inbreds to conditions of habitat rearing. This test consisted of subtracting the absolute value of the hybrid *t* statistic (Row 2, Table 3) from the absolute value of the inbred *t* statistic (Row 1, Table 3). If a habitat effect is large in inbreds but small in hybrids, then scores will be large and positive; if a habitat effect is large in both inbreds and hybrids, the scores will be close to zero; if a habitat effect is large in hybrids but small in inbreds, the scores will be large and negative. The *t* test for the sum of these scores across uncorrelated measures reveals no significant tendency for the scores to be positive ($t = .145$, $df = 7$, $p > .05$). Hence, while inbred and hybrid domestics differ in their pattern of response to habitat environments, these differences are not attributable to superior “developmental homeostasis” among hybrids.

In contrast to the many interactions of genotype with habitat environments, no significant differences emerged in “error” environmental differences within genotypes. An examination of the bottom two rows of Table 3 (Parameters H and I) reveals a remarkable consistency in within-strain environmental variance across inbred and hybrid strains. In all 15 measures tested, the magnitude of within-strain environmental differences in inbreds predicts accurately the magnitude of within-strain environmental differences among hybrids. Thus, hybrids are not demonstrably more resistant to “errors” in environmental rearing conditions than are inbreds.

Relation Between Wildness and Genetic Effects

While the behavioral characters demonstrated several instances of additive (Parameter B), dominant (Parameter C), and heterotic (Parameter D) effects, there was little relationship between these genetic effects and the ability of a character to measure “wildness.” No common source of variance characterized those measures which best separated performances of all domesticated mice from those of all wild mice.

An examination of Table 2 data will reveal that the ranking of a character’s ability to measure wildness (Parameter A, Row 1) is uncorrelated with the measure’s ranking on any genetic effect (Parameters B, C, and D).

The Wilcoxon test failed to indicate any significant relation between the “wildness” parameter A (Table 1, Row 1) and the additive variance parameter B (Table 1, Row 2) ($t = 12$, $df = 10$, $p > .05$). Nor did any significant relationship exist between “wildness” parameter A and the dominance variation parameter, C (Row 3, Table 1) ($t = 25$, $df = 11$, $p > .05$). Neither does any relationship exist between “wildness” parameter A and the heterosis parameter, D (Row 4, Table 1) ($t = 15$, $df = 10$, $p > .05$).

The instances of Genotype by Environment interactions in Table 2 are also statistically unrelated to the character’s ability to measure “wildness.” That is, a character’s ability to separate the mean of all wilds from the mean of all domestics predicts little about the character’s response to habitat effects. For example, behavioral measures that highly characterize wilds vs domestics might also be behaviors that are evolutionarily adaptive in nature. One might, therefore, expect “good” measures of wildness to show higher amounts of developmental homeostasis than “poor” measures of wildness. As a consequence, these adaptive “good” measures of wildness might be protected against variation away from their adaptive norm. An examination of Table 2 reveals that instances of “developmental homeostasis” occur in (a) the best ranked measure of “wildness” (open-field jumping), (b) the worst ranked measure of “wildness” (first day emergence), and (c) mediocre measures of “wildness” (underwater escape and open-field defecation). A Wilcoxon sign test reveals no significant relationship between a measure’s ability to differentiate wildness (Parameter A, Table 2) and occurrence of developmental homeostasis (correlated measures eliminated: $t = 37.5$, $df = 12$, $p > .05$). Nor does examination of Table 3 support the notion that increasing the heterozygosity of domestics increases the similarity of pattern of response of domestics and wilds to environmental habitat. A Wilcoxon test reveals no significant relationship between response of hybrids to habitats (Table 2, Parameter F) and the response of wilds (Table 2, Parameter G) to habitats (correlated measures eliminated: $t = 39$, $df = 12$, $p > .05$). Nor does the response pattern of inbred domestics to habitat (Table 2, Parameter E) resemble that of wilds (correlated measures eliminated: $t = 18$, $df = 12$, $p > .05$).

In summary, data in Table 2 indicate that those measures which most characterize wilds show no systematic pattern of inheritance across domestic stocks. Neither is there a different pattern of response to environmental habitat differences by wilds, inbreds, and hybrids for “good” vs “bad” measures of wildness.

DISCUSSION

The diallel genetic analysis yielded many instances of characters showing additive variance among domestics but few instances of dominance variation or heterosis. Most characters showing additive variation were

open-field behaviors (open-field activity, rearing, grooming, wall seeking, freezing, urination, and defecation). Indeed, jumping was the only open-field behavior not demonstrating significant additivity. The lack of additivity in open-field jumping was probably due to the absence of significant amounts of this behavior among the domestic stocks analyzed (Smith, 1972). Underwater escape (total scores) provided the only other examples of additive variance. Moderate amounts of heterosis were found for only three measures (open-field defecation, open-field activity, and open-field wall seeking). Underwater escape (totals) and wheel activity (totals) provided the only examples of behaviors showing dominance variance. The latter finding of dominance corroborates previous work on the inheritance of wheel activity in domestic mice (Bruell, 1964; Oliverio et al, 1972). The general lack of dominance or heterosis among the 15 behaviors tested corroborates Smith's (1972) conclusion that heterozygosity could account only partially for the differences in behaviors between wild and domestic mice.

Perhaps the most striking finding was the extent and variety of Genotype by Environment interactions. In 14 of the 15 characters tested, laboratory vs naturalistic habitat produced an effect in at least one genetic stock. However, the 15 characters tested failed to provide a single example of a habitat effect which was replicable across all genetic stocks.

Domestication could account for a part of the Genotype by Environment interactions in that wild stocks often react differently to habitat manipulation than did domestic stocks. For example, rearing in a laboratory habitat depressed five behaviors in wilds while exerting no particular effect upon either inbred or hybrid domestics (open-field jumping, open-field defecation, underwater escape—Day 1 and totals, and shock avoidance—Day 1). In contrast, laboratory rearing enhanced open-field urination scores in both inbred and hybrid domestics, while exerting no significant effect on wilds. Finally, laboratory rearing enhanced open-field activity in inbred and hybrid domestics, while severely depressing scores of wilds.

However, domestication did not provide the only source of Genotype by Environment interactions. Hybrid and inbred domestics themselves often differed in their response to habitat manipulations which failed to affect the wilds' performance. Laboratory rearing depressed open-field jumping, open-field defecation, underwater escape (totals), home cage emergence (totals), and shock avoidance (first day) scores for inbred domestics while exerting no significant effects on hybrids. In contrast, laboratory rearing depressed performances of hybrids but not inbreds on two measures (open-field wall seeking and home cage emergence, Day 1). Finally, laboratory rearing enhanced open-field grooming of hybrids, while depressing grooming tendencies of inbreds.

An interesting parallel finding was that "developmental homeostasis" could explain some, but not all, Genotype by Environment interactions among hybrid and inbred domestics. According to the "developmental homeostasis" concept, the developmental system is better buffered, i.e., less influenced by environmental factors, in heterozygotes than in homozygotes. The concept was invoked originally to explain why environmentally induced variability was higher for homozygotes than for heterozygotes in a wide variety of morphological and physiological phenotypes (Lerner, 1954). Indeed, the present study demonstrates several behavioral examples of homozygous inbreds showing greater variability than did heterozygous hybrids. Inbreds responded to laboratory rearing in five behavioral characters (open-field jumping, open-field defecation, underwater escape—totals, home cage emergences—totals, and first day avoidance learning). Hybrids failed to respond significantly to laboratory rearing in these same five characters. However, the present study also demonstrates several counterexamples of inbreds showing less responsivity to habitat rearing than did hybrids. Inbreds failed to respond significantly to laboratory rearing in open-field urination, open-field wall seeking, or home cage emergence—Day 1. Hybrids responded significantly in these same measures. "Developmental homeostasis" cannot explain the latter interactions, nor can it explain instances where heterozygosity changed the direction of habitat effects but not the extent of the effects. Laboratory rearing enhanced the open-field activity and grooming in hybrids but depressed these performances in inbreds.

The present study strongly indicates that the source of environmental variability must be specified before predictions can be made of the relative variability of heterozygotes vs homozygotes. That is, Genotype by Environment interactions occurred for between-habitat environmental differences but not for within-habitat environmental differences. Hybrid and inbred strains failed to differ significantly in within-habitat ("error") variability for any of the 15 behavioral characters. This finding is similar to the lack of differences in "error" variances for inbreds vs hybrids in reversal learning reported by Carran (1972). Moreover, the laboratory vs natural habitats tested represented an entire class of component environmental differences. Hence, a separate analysis must be made of each environment difference in this class before "general rules" can be formulated for Genotype by Environment interactions.

The present study failed to support the notion that "wildness," as measured by its ability in differentiating the performance of wilds from domestics, reflects a unitary genetic trait. The ranking of a behavioral character "wildness" failed to predict the character's pattern of inheritance or response to environmental (habitat) manipulation. This latter result would be expected if a number of different mechanisms had

produced the genetic changes occurring during domestication. Indeed, the sparse available data indicate that artificial selection, natural selection, inbreeding, induction of mutant alleles, and genetic drift can all account for a part of the genetically based behavioral changes occurring during domestication (Connor, 1971; Smith, 1972; Falconer, 1960; MacArthur, 1949; Bruell, 1970). Hence, the authors suggest that future domestication research will involve direct testing of genetic mechanisms responsible for behavioral transformation of feral populations rather than the descriptive analysis of genetic parameters.

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NOTES

1. Inbred strains, which descend from crosses between relatives (brothers, sisters), have a pair of identical alleles at each of their chromosomal loci: A/A, b/b, C/C, d/d—inbred strain 1; a/a, B/B, c/c, D/D—inbred strain 2. Hybrid strains, which descend from crosses between unrelated inbred strains, have a pair of differing alleles at their chromosomal loci: inbred strain 1 by inbred strain 2 = hybrid strain 1 by 2: A/a B/b C/c D/d.

2. Although the simulated natural environment does not contain all the features of all environments in nature, several important characteristics differentiate it from a typical laboratory environment. For example, home cage range is increased, average population density is decreased, and opportunities for extensive agonistic encounters and sexual selection are permitted. Perhaps most important, the physical presence of man is restricted by the availability of cover and handling of the animals is eliminated. These characteristics are not designed to produce a typical wild environment, but rather to approximate the environment of feral *Mus musculus*, which may or may not be typical of other wild environments. Nonetheless, the simulated natural environment contains many of the physical and social characteristics described by Hale (1969) as typifying natural rather than domestication environments.

3. Extrapolating results of a diallel analysis to the population from which the lines were derived is statistically justifiable when at least 10 lines have been tested (Hayman, 1954). Application of the technique to behavioral phenotypes has invariably involved testing fewer than 10 inbred lines. We tested only three lines, and the major basis for extrapolation of results is nonstatistical. First, our three strains were chosen for their ability to bracket the range of behavior over all inbred mouse strains. That these strains would provide performance extremes was based on the investigators' past familiarity with inbred strains. Second, the strains were chosen for their ability to bracket the range of genotypes available, as represented in current laboratory stocks. The three lines were of independent genetic origin and represented three major groups of inbred lines maintained at The Jackson Laboratory (Staats, 1966).

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