Wild mouse open field behavior is embedded within the multidimensional data space spanned by laboratory inbred strains

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The vast majority of studies on mouse behavior are performed on laboratory mouse strains (*Mus laboratorius*), while studies of wild-mouse behavior are relatively rare. An interesting question is the relationship between the phenotypes of *M. laboratorius* and the phenotypes of their wild ancestors. It is commonly believed, often in the absence of hard evidence, that the behavior of wild mice exceeds by far, in terms of repertoire richness, magnitude of variables and variability of behavioral measures, the behavior of the classical inbred strains. Having phenotyped the open field behavior (OF) of eight of the commonly used laboratory inbred strains, two wild-derived strains and a group of first-generation-in-captivity local wild mice (*Mus musculus domesticus*), we show that contrary to common belief, wild-mouse OF behavior is moderate, both in terms of end-point values and in terms of their variability, being embedded within the multidimensional data space spanned by laboratory inbred strains. The implication could be that whereas natural selection favors moderate locomotor behavior in wild mice, the inbreeding process tends to generate in mice, in some of the features, extreme and more variable behavior.

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Laboratory mice are among the most studied animals in behavioral neuroscience and behavior genetics, constituting the primary mammalian model system in the study of the relationship between genomes and phenotypes (Gerlai 2002). Yet, the relevance of their behavior to that of their wild ancestors (*M. musculus domesticus;* Silver 1995; Wade *et al.* 2002) is relatively unknown. Many present day researchers believe that laboratory mice, sometimes also called *M. laboratorius* (Guénet & Bonhomme 2003), display a feeble version of the behavior of their wild progenitors. Laboratory mouse behavior is considered, often in the absence of hard evidence (Ricker *et al.* 1987), to be dull and ‘degenerate’ (Garland 2003), whereas wild-mouse behavior is expected to exceed the behavior of the laboratory strains by far (Austad 2002; Silver 1995), in terms of repertoire richness (Guénet & Bonhomme 2003), magnitude of parameters (Wahlsten *et al.* 2003) and variability of behavioral measures (Koide *et al.* 2000). Laboratory mouse behavior is thus considered to be mostly irrelevant to our understanding of wild behavior, which is in turn thought to represent more faithfully normal behavior. This presumption is examined by us with regard to OF behavior.

Behavior in the OF is a widely used test in genetically engineered mice research (Archer 1973; Bolivar *et al.* 2000; Turri *et al.* 2001). Being a test of unrestrained behavior, it allows the animal to exhibit a wide range of patterns, including walking, running, bounding, progressing, pivoting, circling, horizontal, forward and vertical scanning, as well as rearing, leaning, stretch-attend, grooming and gnawing. This richness makes OF behavior highly suitable for comparative studies of mouse behavior. Except for scoring discrete categories of OF behavior (van Abeelen 1963; 1966; Crusio *et al.* 1989; Crusio & van Abeelen 1986), one can also extract out of it kinematic measures such as location, speed, acceleration, path curvature and heading direction. These measures can subsequently be used to characterize locomotor behavior as well as higher level, cognition-related patterns such as the home-base phenomenon (Drai *et al.* 2001; Drai & Golani 2001) and motivation-related patterns such as wall vs. center behavior (Lipkind *et al.* 2004). The kinematic measures are calculated by us, following a stage of data preparation including smoothing (Hen *et al.* 2004) and segmentation of the path into ethologically relevant building blocks (Drai *et al.* 2000; Drai & Golani 2001; Lipkind *et al.* 2004). These measures span a data space ranging from slow to fast movement, from straight to curved paths, from short to long progression segments, from spatially restricted to spatially spread lingering-in-place episodes, etc. [Kafkafi & Benjamini *et al.* 2005; Lipkind *et al.* 2004; Exploratory behavior surveyed in three laboratories, Mouse Phenome Database (MPD)

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http://www.jax.org/phenome]. Having demonstrated the discriminatory power of these measures in a study performed in three laboratories on eight strains behavior in the OF (A/J, BALB/cByJ, SJL/J, DBA/2J, C3H/HeJ, 129/SvImJ, C57BL/6J and FVB/NJ; Kafkafi & Benjamini *et al*. 2005; Lipkind *et al*. 2004), we can now compare the phenotypes of these strains to that of the local wild mouse (*M. musculus domesticus*) and two commonly used wild-derived strains (CZECHII/EiJ and CAST/EiJ).

Materials and methods

Animals

The eight classical inbred strains in the experiment (A/J, BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, SJL/J and 129S1/SvImJ) are all included in the first priority group recommended for phenotyping by the MPD (http://www.jax.org/phenome) of the Jackson Laboratories. The data of these strains were collected in Tel Aviv University as part of a wider replicability experiment performed in three laboratories (Kafkafi & Benjamini *et al*. 2005). The two wild-derived inbred strains (CAST/EiJ and CZECHII/EiJ) are also recommended for phenotyping by the MPD (groups A and C, respectively). All the inbred groups included 12 male subjects except for the strain CAST/EiJ which included only 10 mice. The wild group included 14 male subjects that were first-generation-in-captivity wild mice caught in a city environment in Israel. All subjects were tested at 9–11 weeks of age.

As it cannot be ruled out that the wild mice used in this study, although belonging to the *M. musculus domesticus* subspecies (Boursot *et al*. 1993), might represent a local subpopulation adapted to a particular environment, the term 'wild mice' is used in this paper merely as a collecting term. The wild-derived inbred strains CAST/EiJ and CZECHII/EiJ were, respectively, derived from a Far-East subspecies, *Mus musculus castaneus* and an East-European subspecies, *M. musculus musculus* (Wade *et al*. 2002). The genomes of the classical inbred strains are mosaics with the vast majority of segments derived from *domesticus* and *musculus* sources and possibly a low presence of segments derived from *castaneus* (through the hybrid *Mus musculus molossinus*; Silver 1995; Wade *et al*. 2002).

Animals were kept in a 12:12 reversed light cycle (Light: 2000 h − 0800 h) and housed two to four per cage (except for the SJL/J mice that were singly housed because of their high aggressiveness) under standard conditions of 22 °C room temperature and water and food *ad libitum*. The animals were housed in their room for at least 2 weeks before testing. All animals were maintained in facilities fully accredited by NIH Animal Welfare Assurance Number A5010-01 (TAU). The studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH, 'Principles of Laboratory Animal Care' (NIH publication no. 86–23, 1996).

Experimental protocol

The experiment was performed in an ethological OF test setup with a large (250 cm diameter) circular arena having a non-porous gray floor and bordered by a 60-cm high, primer-gray painted, continuous wall. Several landmarks of various shapes and sizes were attached in different locations to the arena wall and to the walls of the room where the arena was located. The arena was illuminated with two 40-W neon bulbs on the ceiling, above the center of the arena. The experiments were conducted during the dark part of the cycle, 1–2 h after its onset. Each experimental animal was brought from its housing room to the arena in a small opaque box and placed within it in a standardized location, near the wall, while still in the box. After 20 seconds, the box was lifted, and a 30-min session began. The arena was recorded using a resolution of 25 samples per second and approximately 1 cm. The animals' movement was tracked using Noldus EthoVision® automated tracking system (Noldus *et al*. 2001).

The SEE analysis

Tracking procedures and path analysis using SEE, a software-based Strategy for Exploring Exploration (SEE; Drai & Golani, 2001; Kafkafi 2003), were as detailed in previous phenotyping studies (Kafkafi *et al*. 2003; Kafkafi & Benjamini *et al*. 2005). The behavior was quantified using 27 measures or 'end-points' (listed in Table 1 and explained in the *Supplementary material*), most of them simple properties of lingering and progression segments, such as their number, their length, their maximal speed etc., and some more complex, quantifying wall vs. center behavior (Lipkind *et al*. 2004) and the strategy of occupancy of the arena (the home-base phenomenon; Drai *et al* 2001).

The raw data obtained from the tracking system were smoothed using a specialized algorithm implemented in the stand-alone program 'SEE PATH SMOOTHER' (Hen *et al*. 2004). This procedure produces reliable estimates of momentary speeds during motion (momentary speeds during arrests are defined as zero).

As has previously been shown, rodent locomotor behavior consists of two distinct modes of motion – progression segments and lingering episodes (Drai & Golani 2001; Golani *et al*. 1993). During progression segments, the animals traverse relatively large distances attaining relatively high speeds. During lingering episodes, the animals stop and perform scanning movements, while staying in a circumscribed neighborhood. Segmentation of the smoothed path into progression segments and lingering episodes was done using the EM algorithm (for explanation see Everitt 1981) with a two-Gaussian mixture model (Drai *et al*. 2000). Stand-alone user-friendly software for smoothing (SEE PATH SMOOTHER) and for segmentation (SEE PATH SEGMENTOR) can be downloaded at http://www.tau.ac.il/~ilan99/see/help.

The development of the new algorithms and end-points described in the *Results* was done using the Mathematica-

Table 1: Behavioral end-point values in the wild mice

No.	End-point name	Wild-mouse normalized median values	Wild-mouse normalized IQR values
1	Wall ring thickness	0.12	0.05
2	Latency to maximal half speed	0.19	0.03
3	Median inter-minima interval	0.80	0.31
4	Q95 progression segment maximal speed	0.66	0.21
5	Q95 Length of progression segments	0.72	0.65
6	Median segment acceleration to maximal speed	0.81	0.58
7	Median radius of turn (fixed time interval)	0.67	0.73
8	Home-base relative occupancy	0.06	0.12
9	Proportion of resting at home base	0.16	0.16
10	Dart	0.41	0.90
11	Number of stops per distance	0.07	0.06
12	Activity proportion of lingering	0.07	0.10
13	Time proportion of lingering	0.37	0.87
14	Median duration of lingering	0.32	0.17
15	Median spatial-spread of lingering	0.12	0.51
16	Q75 lingering maximal speed	0.34	0.65
17	Relative activity decrease	0.25	0.02
18	Median curvature (fixed distance interval)	0.18	0.15
19	Center rest proportion	0.44	0.12
20	Proportion of lingering time away from wall	0.59	0.17
21	Number of progression segments	0.80	0.22
22	Percentage of arena occupancy	0.41	0.30
23	Center activity proportion	0.58	0.16
24	Lingering mean speed	0.40	0.65
25	Q95 Duration of progression segments	0.70	0.43
26	Distance traveled (activity)	0.59	0.74
27	Lingering-progression segments threshold speed	0.85	0.24

The presented values are the normalized medians and normalized Inter Quartile Ranges (IQRs) of the wild mice, relative to all the other tested strains. In each end-point, the lowest score was set as 0 and the highest as 1. All the other scores were normalized, respectively, within the new 0–1 interval. The wild group did not present extreme behavior in any of the end-points, both in terms of medians and in terms of IQRs of the end-points values. The order of the end-points is identical to the order presented in Figs 1,2(a) and 3(a). For explanation of end-points see *Supplementary material.*

based program SEE Package (Drai & Golani 2001) and two extension programs, 'SEE EXPERIMENT EXPLORER' and 'SEE ENDPOINT MANAGER' (Kafkafi 2003).

Statistical methods

For all end-points, the appropriate transformations of the variables were chosen so that the distribution within strains would be quite close to the normal distribution and the variances would be homogeneous across genotypes. Hierarchical clustering of the variables for the purpose of choosing their order on the polygon was done using the 'CorrelationMatrix' function in MATHEMATICA™ software (Wolfram 2004).

Results

To compare the phenotypes of wild mice to that of the 10 inbred strains, we visualize the median values of the 27 calculated end-points, by using a polygon plot for each of the 11 strains (Fig. 1). In this type of icon plot, the distance from the center of the icon to consecutive corners of the polygon represents relative values of selected variables. Data points plotted in the centermost part represent low end-point values, and data points plotted in the outermost part represent high values. The order of end-points in the figure was determined by applying a hierarchical correlation test over the end-points. The polygon thus highlights the unique data subspace occupied by a strain within the overall data space of OF behavior.

Before making the claim that wild-mouse behavior is moderate, we familiarize the reader with our high-dimensional representation of OF behavior in the laboratory strains. Behavioral profiles vary considerably among strains. In A/J, for example, the polygon consists of minimal values in end-points representing progression and speed (1–7; 19–27) and maximal values in end-points related to lingering behavior (11–13). In contrast, the FVB/NJ icon consists of maximal

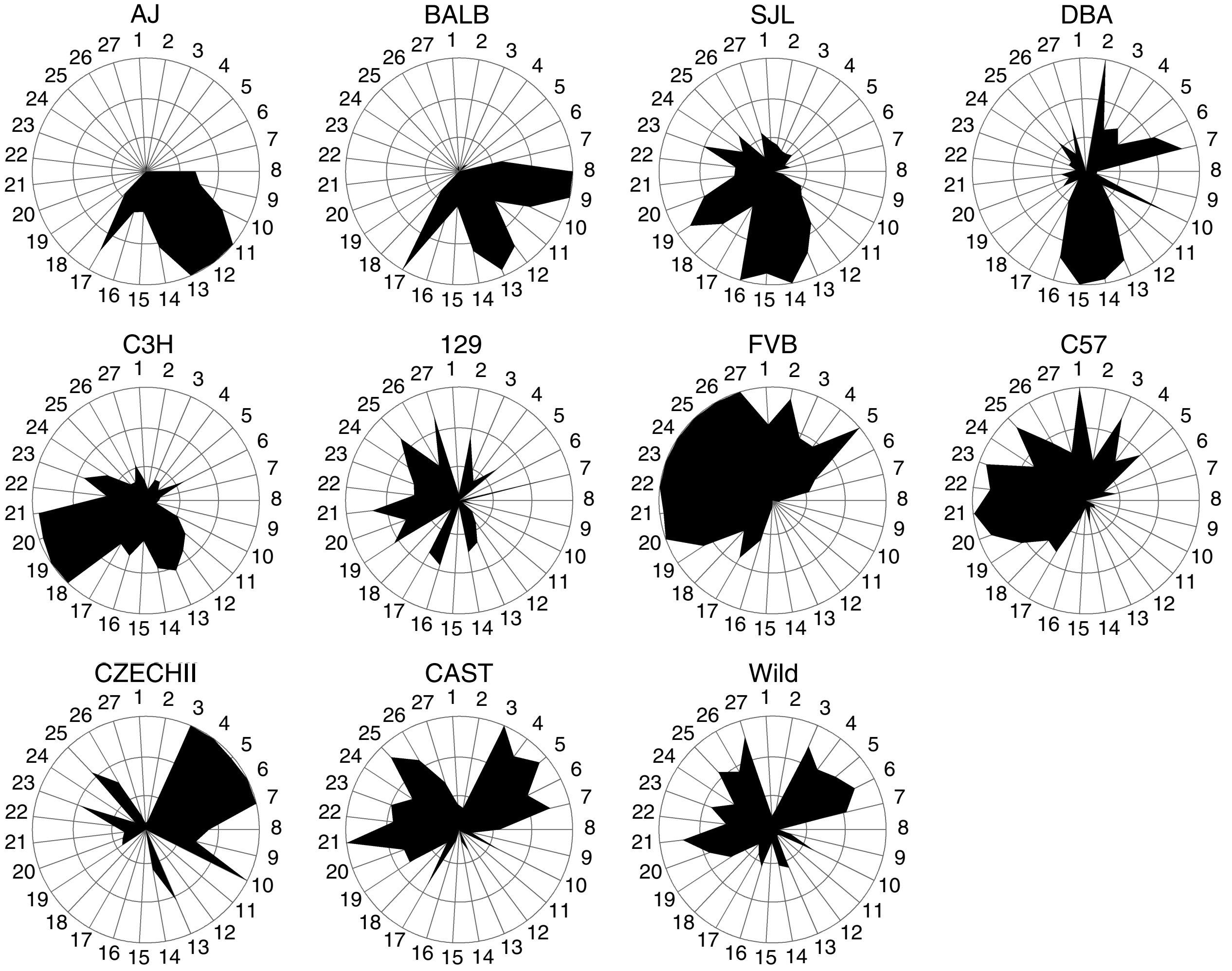
AJ, BALB, SJL, DBA, C3H, 129, FVB, C57, CZECHII, CAST, Wild

Figure 1: Median values of 27 behavioral measures are visualized using a polygon plot for each of the 11 strains. A separate polygon icon is plotted for each strain; relative values of the selected variables for each strain are represented by the distance from the center of the icon to consecutive corners of the polygon, with minimal values in the center and maximal values on the perimeter (variables are numbered, and presented in a clockwise order, in correspondence with the measures listed in Table 1). The polygon thus highlights the unique profile occupied by a strain within the overall data space of open field behavior. The wild-mouse profile occupies a moderate position within a diversity of *Mus laboratorius* profiles.

values in end-points related to progression and activity (5 and 20–27) and minimal values in end-points related to staying-in-place behavior (11–15) including staying at home base (8–9).

The multiplicity of activity-related end-points and lingering-related end-points is not redundant: most of the end-points add information on strain-specific differences. DBA/2J, SJL/J and C3H/HeJ, for example, share the same relatively low values of distance traveled (26), yet the very same distance is covered in C3H/HeJ by performing significantly more progression segments in comparison with SJL/J and DBA/2J (21). In addition, C3H/HeJ mice perform short, and SJL/J and DBA/2J long, lingering episodes between progression segments (14). Similarly, although FVB/NJ is higher than CAST/EiJ and CAST/EiJ is higher than CZECHII/EiJ on activity (26), CZECHII/EiJ scores highest on speed among these strains and FVB/NJ lowest (4), i.e. speed and activity are not redundant in relation to each other. Finally, although both C3H/HeJ and FVB/NJ perform a similar number of progression segments (21), they segregate on activity (26).

The icons highlight both overall similarities and specific differences between strains*:* both A/J and BALB/cByJ score high on staying-in-place-related aspects of behavior (e.g. 12–14) and on activity increase along the session (17). BALB/cByJ, however, also score high on staying-at-home-base behaviors (8, 9). Likewise, the wild group and wild-derived CAST/EiJ share an overall similarity but differ from wild-derived CZECHII/EiJ on, for example, Dart (10, Kafkafi

2003) and on the proportion of lingering duration, which is brought about by a gradual increase in the duration of lingering episodes performed by CZECHII/EiJ across the session (13). CZECHII/EiJ, in contrast to the wild mice and CAST/EiJ, is characterized by a low number of progression segments (21) and by a strong decrease in activity along the session (17).

We now turn to wild-mouse OF behavior and immediately note that it is moderate in terms of end-point values in comparison with the phenotypic range covered by the 10 inbred strains. By projecting all the data points of Fig. 1 into a single disk (Fig. 2a), one can rank a focal end-point value in relation to the values of the other strains in that end-point. For example, within the wild and wild-derived strains, CZECHII/EiJ (▽) is the least active (26) and CAST/EiJ (+) the most active, and the wild mice (●) are situated in the middle between them. Contrary to a wide-held view, the wild mice occupy neither the highest nor the lowest position in any of the end-points defining the variable space (Fig. 2a). This is highlighted in Fig. 2(b) which plots the number of variables in which a strain exhibited an extreme (highest or lowest) phenotype. Furthermore, no individual wild mouse exhibited an extreme value in any of the end-points: there were always inbred mice that surpassed it. This means that in the context of OF behavior of the 10 inbred strains, these wild *M. musculus domesticus* representatives do not expand the phenotypic range in any of the 27 end-points. In contrast, the two wild-derived strains do exhibit several extreme values.

To examine whether the moderateness in end-point values of wild mice is a misleading outcome obtained by extremely high-spread values, we calculated for each end-point the Inter Quartile Range (IQR), which estimates phenotypic spread within each end-point, for each of the strains. As shown in Fig. 3, the wild group did not show an extreme IQR in any of the end-points. For each, there were always inbred strains that presented higher or lower behavioral spread than the wild mice. While CAST/EiJ presented extreme values in seven end-points, and CZECHII/EiJ in five, the wild mice presented none (Fig. 3b).

Discussion

In this study, we compared the OF behavior of a wild group of mice to that of 10 inbred strains, using 27 kinematic measures reflecting locomotor, cognition-related and motivation-related aspects. The comparison revealed that wild-mouse behavior is moderate in terms of end-point values, being embedded within the data space spanned by the inbred strains. In contrast to the wild mice, which are never extreme, each of the inbred strains displays extreme values: FVB/NJ, for example, is the highest on distance traveled, and A/J is the lowest on it. C3H/HeJ is the highest on path curvature, and CZECHII/EiJ is the lowest on it. DBA/2J is the highest on the spatial spread of

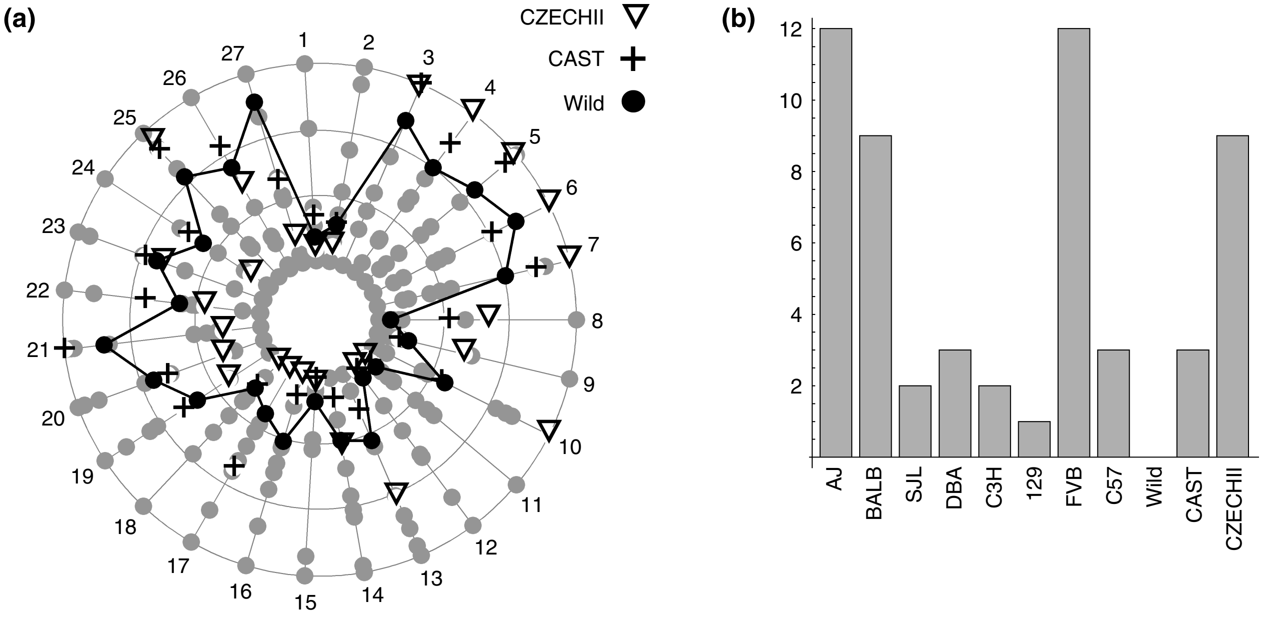

Figure 2: (a) A projection of all end-point values (median values of all strains) into a single disk. Values of wild mice are presented as black dots (●) and connected by lines that restore the icon depicted in Fig 1. The wild-derived strains are presented as down-triangle marks (▽) for CAST/EiJ and as plus marks (+) for CZECHII/EiJ. All other strains are presented as light-gray dots (●). Note the moderate values characterizing the wild mice (medians presented in Table 1). (b) The number of extreme (lowest or highest) end-point values in each strain. The wild mice presented no extreme values.

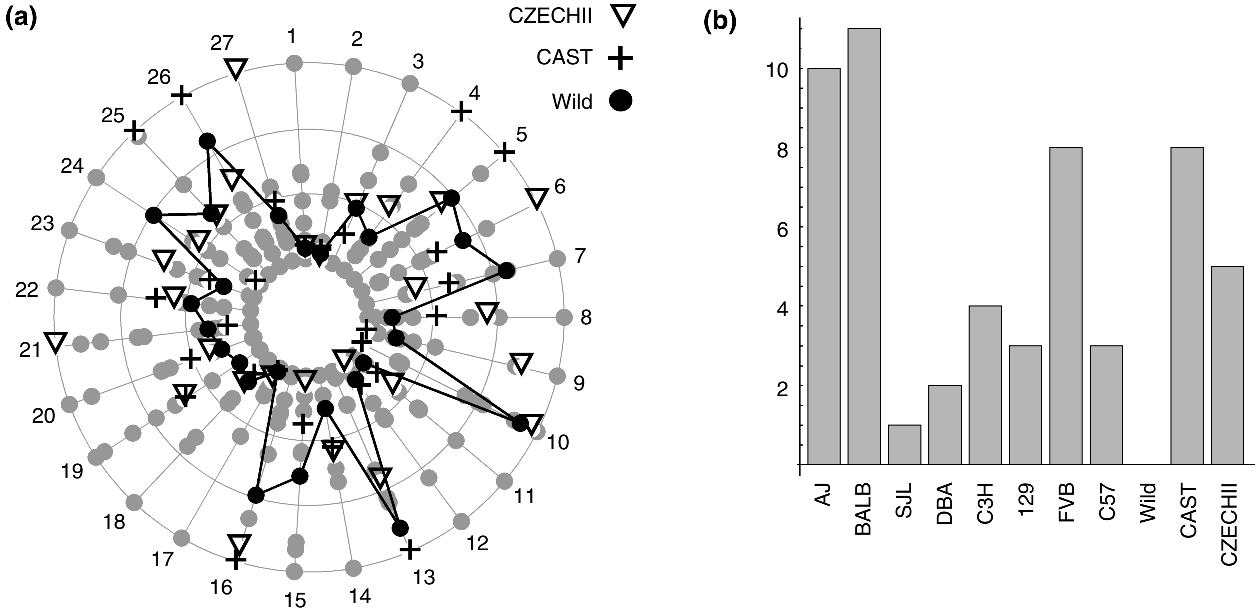

Figure 3: (a) A projection of all normalized Inter Quartile Range values (IQRs) of all strains into a single disk highlights the moderate dispersal estimates in the wild mice. The wild mouse data points are connected by lines. The order of the variables is identical to that presented in Figs 1,2(a) and Table 1. For each of the end-points, there were always inbred strains that presented higher and lower dispersal estimates than the wild mice. (IQR values presented in Table 1). (b) The number of end-points in which extreme dispersal estimates (lowest or highest IQRs) were exhibited by each of the strains. The wild mice did not present extreme dispersal in any of the end-points, whereas CAST/EiJ presented extreme estimates in seven end-points and CZECHII/EiJ in five.

lingering episodes, and CAST/EiJ is the lowest on it. CAST/EiJ performs the highest number of progression segments, and BALB/cByJ performs the lowest number. This regularity applies to all the 27 variables: the wild mice always occupy an in-between position between two extreme strains. In this sense, each one of the inbred strains portrays a 'caricature' of wild-mouse behavior, in much the same way that a Pincher or a Great Dane portray size-caricatures of the wolf, thus uncovering the wolf's phenotypic potential. Here, we show that several such 'caricatures' cover the whole range exhibited by their wild progenitor and more. Thus, as far as OF behavior is concerned, the study of even a few inbred strains suffices to provide a rough notion of what wild-mouse behavior consists of.

The wide phenotypic inter-strain range covered by inbred and other commonly used laboratory strains has also been demonstrated in the water-maze setup, where the wide range of average escape times across these strains, reflecting spatial memory, has been shown to be equivalent to the range observed across mouse preparations with severely impairing mutations (Lipp & Wolfer 2003). These authors point out that inbred strains are, in general, likely to show extreme behavioral traits equivalent even to strong anomalies. The extreme end-point values characterizing some of the inbred strains in our study could also be explained by the absence of selective forces that exist in the wild, which could cause phenotypic drift in inbred strains (Silver 1995).

We do not doubt that in certain situations, wild-mouse behavior exceeds by far the behavior of inbred strains in terms of repertoire richness and magnitude of end-point values. These situations, however, typically involve 'risk assessment,' escape attempts from aversive environments (Augustsson & Meyerson 2004; Blanchard *et al.* 1998; 2001; Hendrie *et al.* 1996; Lipp & Wolfer 2003) and resistance to handling and to recapture (e.g. Holmes *et al.* 2000; Wahlsten *et al.* 2003). All these 'fight and flight' behaviors are reduced by inbreeding (Connor 1975) and by domestication (see Koide *et al.* 2000; McPhee 2003; Price 2002).

As elaborated by Garland *et al.* (1995), some biologists presume laboratory strains to be 'degenerate' in one or more ways (Dohm *et al.* 1994; Richardson *et al.* 1994). As far as OF behavior is concerned, our comparison reveals that rather than extending the domain of obtained phenotypic values in a 'degenerative' direction, inbreeding and non-directed domestication extend it in multiple directions.

A recently performed multiple strain comparison (Kafkafi & Benjamini *et al.* 2005) reveals that the end-points obtained in the OF test cover a wide range of values. Therefore, moderate results do not reflect an insensitivity of the SEE analysis tools. For example, activity ranges between median values of 36.4 m (in A/J) and 421.0 m (in FVB/NJ), whereas wild mice have a median value of 263.6 m. In addition, for most end-points, the range within strains is much smaller than the overall range covered in that end-point by all strains together.

In other words, the end-point values are highly discriminative between strains. This test is therefore very sensitive to the characteristics of locomotor behavior.

Except for the moderateness of the end-point values in the wild mice, we also show that the wild mice did not present extreme dispersal estimates in any of the end-points. In other words, in each of the 27 locomotor and exploratory end-points, there were always inbred strains that presented either lower or higher behavioral spread than the wild mice. While the instances of lower spread, observed in the inbred strains, can be explained by a lower genetic variability, a theory that could explain the higher spread instances has been proposed by Waddington (1940; 1942; 1957). The theory proposes that in the wild, during development, selective forces canalize (or buffer) adaptive patterns that are important to fitness, against disruptive environmental influences. Following Waddington's ideas, it has been suggested that in the wild, developmental homeostasis, and hence the stability of species-specific traits, is brought about by genomic mutual adaptation (coadaptation involving coadapted heterozygosity in complex polygenic systems, coadapted homozygosity and coadapted interlocus interactions; Lerner 1961). Returning to mice, an indication that genetically heterogeneous random-bred mice may sometimes be less variable than inbred material was obtained long ago by Emmens (1939). Using the argumentation presented above, Robertson and Reeve (1952) and Rasmuson (1952) (both quoted in McLaren & Michie 1954), explain the reduced phenotypic variability of mice having a mixed background by suggesting that 'the more heterozygous individuals, will carry a greater diversity of alleles, and these are likely to endow them with greater biochemical versatility in development. This will lead to ... a reduced sensitivity to environmental variations, since there will be more ways of overcoming the obstacles which such variations put in the way of normal development.' Lipp and Wolfer (2003) use the same arguments in relation to the behavior of inbred mice vs. mice of mixed background and F1 hybrids.

The demonstration that for each of the 27 examined locomotor and exploratory end-points there were always inbred strains that presented higher behavioral spread than the wild mice supports the recommendation already made half a century ago, to complement experiments made on inbred strains with experiments on inter-strain F1 hybrids. Such material combines the genetic uniformity of inbred strains with the 'buffering' action against environmental variations which heterozygosity exerts (McLaren & Michie 1954).

M. musculus musculus and *M. musculus castaneus* are genetically roughly equidistant from *M. musculus domesticus* (Wade *et al*. 2002). Also, there are no geographical interaction zones, and presumably no gene flow, between *domesticus* and *castaneus*, whereas *domesticus* and *musculus* are sympatric along a thin stripe in Europe, and *Castaneus* and *musculus* are sympatric along a broader zone in Eastern Asia (Silver 1995). It is therefore interesting to note that as far as OF behavior is concerned, the wild *domesticus* group and the CAST/EiJ strain present an overall similarity (see icons in Fig. 1), whereas the CZECHII/EiJ strain stands out as different from both the wild and the CAST/EiJ mice. Specifically, the distance traveled by the wild mice and CAST/EiJ is higher (respectively 264 m and 310 m) than that traveled by CZECHII/EiJ (225 m); unlike its counterparts, CZECHII/EiJ starts with high activity and then gradually dwindles, ending in long periods of staying in place. CZECHII/EiJ is also the fastest of the three (and faster in this setup than all the other examined strains; Quantile 95 above 100 cm/second), tracing paths of the lowest curvature compared with the other two, whose paths' curvature is similar. CZECHII/EiJ is also the only strain bounding in this arena (along the wall, in spurts of up to 16 m at a time, at a speed of 90 cm/second, lower than its maximal running speed). During lingering episodes, it is, however, the slowest. Unlike the other two strains, CZECHII/EiJ's speed in the center is much higher than its speed along the wall, and unlike the wild mice, the two wild-derived strains perform abrupt large shifts of direction during incursions to the center (medians of 46 degees for wild, 58 degrees for CZECHII/EiJ and 62 degrees for CAST/EiJ). While all three are wall-huggers, performing the majority of their activity within a relatively thin ring along the wall, CZECHII/EiJ's ring is the thinnest.

In conclusion, the striking diversity of phenotypes among domestic mouse strains, which is only matched by that observed among species in nature, offers a unique resource for following the changes from wild species to domestic strains. The side-by-side study of domestic mice and their wild congeners provides each other's behavior with a useful perspective. It would be useful to follow this study with a comparative study including the wild *domesticus,* wild *musculus* and wild *castaneus* subspecies, each collected in a variety of environments and locations, in order to represent the degree and nature of adaptive variation in this species. The spectrum of phenotypes of wild-derived and additional inbred mouse strains and their F1 descendants will provide insights into the process of phenotypic evolution, as it is manifested in domestication.

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Supplementary material

Appendix S1. The supplementary material contains definitions of all the 27 behavioral end-points used in this work.

This material is available as part of the online article from http://www.blackwell-synergy.com

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