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Adult Hippocampal Neurogenesis in Natural Populations of Mammals

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THIS CHAPTER IS BASED ON THE PREMISE that if we are to acquire a deep understanding of adult neurogenesis—what it is selected for (i.e., its functional and adaptive significance), what causes it to go up or down (e.g., species constraints, reproductive hormones, seasonality, stress, and environmental conditions), and why it declines with age—the research must ultimately be grounded on an evolutionary and ecological foundation. The aphorism of Dobzansky (1973) is particularly apropos: "Nothing in biology makes sense, except in the light of evolution." Thus, simply focusing on humans and those laboratory species we select for will not be sufficient to crack this enigma. Such a deep understanding may also aid in ameliorating debilitating aspects of the human condition after injury or in disease. This chapter advocates for studies that deal with animals that live out their lives in the context of what they were actually selected to do. Given the paucity of studies from nature, it raises more questions than it answers. It focuses largely on mammals.

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The formation of new neurons in adult animals is a highly conserved trait in vertebrates, occurring in all groups, from fish to mammals in various brain regions. It is linked to a diversity of life history traits such as lifelong body growth in fishes and rats and seasonal variation in song control nuclei in birds (Lindsey and Tropepe 2006). In mammals, adult neurogenesis occurs physiologically in two germinal areas: the subventricular zone (SVZ), which lies adjacent to the lateral wall of the lateral ventricle and generates GABAergic olfactory bulb neurons, and the subgranular zone (SGV) of the dentate gyrus of the hippocampal formation. The critical question in understanding adult hippocampal neurogenesis is why the mature neural synaptic connections may not be sufficient to allow animals to cope with their environment. What memory or cognitive need(s) dictates the continual generation of new neurons in adults that simple rewiring or strengthening of the connections cannot?

The study of adult neurogenesis in natural populations has been essentially ignored. Birds (~9900 extant species) originated from reptiles and have been separated for approximately 300 million years from the line that eventually led to mammals (~5400 extant species). Thus far, our understanding how neurogenesis functions in free-living animals in the natural world has been restricted to only one bird species (black-capped chickadees; Barnea and Nottebohm 1994) and a few mammal species as described in detail below. For the rest, all adult neurogenesis studies target either species bred and raised specifically for the laboratory (rodents) or in the laboratory (various primates) or domesticated animals. However, even these latter studies are not frequent.

Mice and rats have been enormously useful models to understand the molecular and biochemical basis of the regulation of adult neurogenesis and their implications for behavior (Lledo et al. 2006). They offer the degree of environmental control and genetic homogeneity that may be needed to limit experimental variation. However, findings from the laboratory may be of limited applicability as to why adult neurogenesis occurs in the first place, and thus what it is selected for, both in wild species functioning in their natural environment and perhaps in humans.

First, laboratory rodents may be suboptimal models to understand what happens in the natural world, as the selective regimes laboratory animals experience are significantly different from those experienced by animals in nature (Künzl et al. 2001; Wolff 2003). Laboratory rodents are often less aggressive, less aware of their environment, explore less, are more social, and respond more to stressors than their natural counterparts. Indeed, based on an analysis of aging in laboratory and natural populations of the house mouse, it has been concluded that laboratory-adapted stocks of rodents may be particularly inappropriate for the analysis of the

genetic and physiological factors that regulate aging in mammals. Findings derived from them may be unable to provide much information about the mechanisms of aging in mammals, except under the highly artificial conditions of the laboratory (Miller et al. 2002). The same may apply to the study of adult neurogenesis.

Second, at the species level, adult neurogenesis may have a central role in evolutionary adaptations to dealing with particular ecological pressures, and differences among species may be essential to life history adaptations (Wingfield and Sapolsky 2003; Boonstra 2005; Smulders 2006). Ecological and evolutionary complexity in the natural world may result in a diverse array of physiological solutions to ecological problems. Thus, one set of guiding generalities to explain the role of neurogenesis and how it functions may not fit all avian and mammalian life histories. In particular, mammals span the gamut of life history variation, with species at one end of the spectrum having high reproductive rates, rapid development, and short life spans (e.g., rats and mice) and those at the other end having the opposite traits (e.g., bats, marmots, elephants, deer, some carnivores, and long-lived primates such as humans). Species with the former traits have been studied heavily to gain insights into the functioning of neurogenesis. In contrast, species with the opposite suite of life history traits have not been well-studied, and thus neurogenesis may operate in a different mode. Therefore, it is critical to also study species that span both the phylogenetic spectrum and the gamut of longevity from short-lived to long-lived species.

Third, at the individual level, adult neurogenesis may be the template that allows animals to cope with variation in their environment—the environmental certainty and uncertainty that are the daily fare of existence of all organisms (e.g., winter vs. summer, times of low vs. high social pressure, times of low and high predation pressure, and times of severe vs. benign environmental conditions).

The key to understanding the role that adult hippocampal neurogenesis may have in memory, and thus in predicting the future, is the inherent time lag between the production of an incipient neuron and its integration as a fully functional neuron with dendrites and axons. Such a lag typically takes 2–4 weeks in rodents (Piatti et al. 2006) and approximately 5 weeks in monkeys (Ngwenya et al. 2006). Thus, if adult neurogenesis has fitness consequences, birth of new neurons must be anticipatory, i.e., related to future, not present, needs. These needs may give insight into the adaptive purpose of neurogenesis. Thus, do rates of neurogenesis vary over the biological year (i.e., most animals have distinct breeding and nonbreeding seasons)? Is it related to particular behavioral and cognitive demands?

IS ADULT HIPPOCAMPAL NEUROGENESIS A FEATURE COMMON TO MAMMALS?

Presence in the Order Rodentia

Reports on adult neurogenesis in wild-living mammals are scarce, and the methods are not always comparable. In many studies, performed prior to widespread application of the bromodeoxyuridine (BrdU) method in the early 1990s, the evidence is only suggestive and would not stand the scrutiny of today's requirements. More recent reports are generally more reliable, but they need to be interpreted with caution, because the range of phenotypic markers is limited. Neurogenesis in the hippocampus of wild yellow-necked wood mice (*Apodemus flavicollis*), wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), and European pine voles (*Microtus subterraneus*) has been visualized immunohistochemically using markers against Ki-67 (a protein active during mitosis), as well as Doublecortin and NeuroD (both found in neuronal lineage precursor cells and/or young neurons) (Amrein et al. 2004b). In wild American meadow voles (*Microtus pennsylvanicus*), proliferating cells were labeled by incorporation of injected [^{3}H]thymidine (Galea and McEwen 1999). In Eastern gray squirrels (*Sciurus carolinensis*) from the United States, proliferating cells were found after injection of BrdU, subsequently incorporated in the DNA of dividing cells (Lavenex et al. 2000). In a study comparing wild yellow-pine chipmunks (*Tamias amoenus*) and Eastern grey squirrels from Canada, cell proliferation and neuronal fates were visualized immunohistochemically using Ki-67 and Doublecortin (Barker et al. 2005). Preliminary data in tropical bats (Chiroptera) from South America and Africa obtained by immunohistochemical analysis with markers against Ki-67, MCM2 (a marker for nonactive precursor cells), Doublecortin, and NeuroD showed sparse or no adult hippocampal neurogenesis (Amrein 2005; Amrein et al. 2006).

Investigations in domesticated and laboratory-bred mammals other than mice and rats reported adult neurogenesis in the hippocampus of several rodent species. In guinea pigs (*Cavia cavia*), Altman and Das (1967) were the first to show postnatal cell proliferation using [^{3}H]thymidine for visualization, whereas later studies also included adults using BrdU in combination with a double-labeling technique discriminating neurons from glia by means of NeuN (a marker for neurons) and GFAP (glial fibrillary acid protein) (Guidi et al. 2005). In gerbils (*Meriones unguiculatus*), adult neurogenesis was documented with BrdU (Dawirs et al. 2000), and in prairie voles (*Microtus ochrogaster*) by using BrdU-combined double and triple labeling with GFAP, Map-2, NeuN, and TuJ1 (Fowler et al. 2002).

Photoperiod-dependent adult neurogenesis in various brain regions of golden hamsters (*Mesocricetus auratus*) was described by Huang et al. (1998) using markers for BrdU in combination with NeuN and GFAP.

Presence in the Other Mammalian Orders

The presence of adult hippocampal proliferation and neurogenesis has been indicated in rabbits (*Oryctolagus cuniculus*, order Lagomorpha), using [^{3}H]thymidine (Gueneau et al. 1982) or immunohistochemical markers against the M1 subunit ribonucleotide reductase (RNR, a rarely used proliferation marker), GFAP, calbindin, neurofilament, and nestin (Zhu et al. 2003). With the same protocol, Zhu et al. (2003) found adult neurogenesis in pigs (*Sus domesticus*, order Artiodactyla) and sheep (*Ovis aries,* order Artiodactyla). Tree shrews (*Tupaia glis*, order Scandentia) were investigated using BrdU and neuron-specific enolase (NSE) (Gould et al. 1997). In marsupials, there is a report for one species, the small, mouse-like fat-tailed dunnart (*Sminthopsis crassicaudata*, order Dasyuromorphia), based on [^{3}H]thymidine incorporation and immunohistochemistry for GFAP, PSA-NCAM, and calbindin (Harman et al. 2003).

In nonhuman primates, adult hippocampal neurogenesis was observed in rhesus and cynomolgus monkeys (*Macaca mulatta* and *Macaca fascicularis*, order Primates) by administration of BrdU in combination with extensive immunohistochemistry (TOAD-64, calbindin, NSE, GFAP, PCNA, O4, CNP, NeuN, and TuJ) (Gould et al. 1999; Kornack and Rakic 1999). In Japanese macaques (*Macaca fuscata*), BrdU was used in combination with markers for Musashi1, Nestin, NeuN, β-tubulin class III, GFAP, S100β, CNP, and GAD (Tonchev et al. 2003). Adult neurogenesis has also been reported for the New World marmoset monkey *Callithrix jacchus*, using BrdU combined with immunohistochemistry against the neuron-specific enolase NSE (Gould et al. 1998). In comparison to rodents, monkeys show markedly lower basal proliferation rates and a lower number of cells taking neuronal fate (Tonchev and Yamashima 2006), as well as much longer maturation times of the newly born cells (Ngwenya et al. 2006).

To summarize, of 25 mammalian orders, adult hippocampal neurogenesis of variable degrees has been investigated and reported in only six orders (except for rodents, all orders with few or only one species), whereas one order (order Chiroptera) revealed thus far no adult neurogenesis for the majority of species investigated (only 3 species of 16 showing sparse neurogenesis and the others none) (Amrein 2005; Amrein et al. 2006 and unpubl.). The remaining 18 mammalian orders have not been investigated. Moreover, in many studies, quantification is missing, incomplete,

or anecdotal. Obviously, one may assume but, cannot ascertain, that adult hippocampal neurogenesis is a widespread mammalian trait. In addition, the diversity of techniques has thus far hampered a systematic comparison of neurogenesis rates in the species investigated. Nevertheless, some important findings have emerged.

AGE DEPENDENCE OF NEUROGENESIS

Thus far, the most common finding in all species investigated for this trait is an age-dependent decline in adult hippocampal neurogenesis. Protracted neurogenesis of granule cells peaks at puberty and declines steadily thereafter, as documented in mice, rats, and monkeys (Gould et al. 1999; Kempermann et al. 1998; Kuhn et al. 1996; Seki and Arai 1995), albeit with considerable species differences. In wild species, age determination is never as precise as in laboratory species and is generally restricted to age classes, such as juvenile, adult, or old. Nonetheless, it has been clearly shown that older wood mice, voles, chipmunks, and squirrels show a decline in ongoing proliferation compared to that of young and adults (Amrein et al. 2004b; Barker et al. 2005). Thus, the decline in ongoing proliferation activity in elderly laboratory-bred animals is not a domestication effect but appears to be a truly general phenomenon, probably occurring in humans as well (Fahrner et al. 2007).

INDIVIDUAL AND SEX DIFFERENCES OF ADULT NEUROGENESIS IN WILD POPULATIONS

Seasonal reproduction is the norm for most species in the wild (Bronson and Heideman 1994). Wild rodents show a recognizable effect of hormonal fluctuations on adult neurogenesis. Seasonal fluctuation in proliferation activity due to reproductive state has been reported in wild-trapped female meadow voles, whereas nonproductive females with low levels of estradiol showed a higher number of proliferating cells (Galea and McEwen 1999; Ormerod and Galea 2003). These findings were replicated in the laboratory, where manipulations of hormone levels in male and female voles revealed different effects on proliferation and survival of newborn cells (Ormerod and Galea 2001; Ormerod et al. 2004), an effect that appeared also to be moderately correlated with spatial memory in water-maze tasks (Ormerod et al. 2004). Furthermore, reduction of daylight length doubled the rate of adult neurogenesis in hamsters (Huang et al. 1998). Whereas wild house mice breed seasonally (Berry 1981), laboratory mice and rats reproduce continually due to defective melatonin

processing (Kennaway et al. 2002), thus evidence for a seasonally cycling hormone level and corresponding proliferation regulation is unavailable. However, the effect of manipulated hormone levels on adult neurogenesis of laboratory rodents is well-documented (for a review, see Galea et al. 2006).

Whether cell proliferation, survival, and fate of the new cells in wild rodents can be altered with experimental factors other than hormones (i.e., activity, diet, and learning tasks) is still unknown. Lavenex et al. (2000) investigated whether the basal proliferation activity in wild squirrels shows seasonal variations correlated with their caching activities, but they failed to find such fluctuations.

ADAPTIVE SIGNIFICANCE AND FUNCTION OF ADULT NEUROGENESIS ACROSS WILD LIVING SPECIES

The central motivating factor for studying adult hippocampal neurogenesis in natural populations has been the putative (and still debated) beneficial role of newly born granule cells on spatial learning and memory performance of rodents (**Abrous and Wojtowicz, chapter 21 in this book**). If the observation that adult rats generate 10,000 new neurons daily (Cameron and McKay 2001; McDonald and Wojtowicz 2005) can be extended to most other mammal species, it is highly unlikely that this trait is some vestigial holdover from the past, as all animals are living in an energy-limited world. Thus, one would expect to find regulated levels of neurogenesis in species with differential demands for memory abilities. To some extent, these expectations have been met.

Barker et al. (2005) studied chipmunks and squirrels with differential food-caching strategies that prepare them for winter. Chipmunks have small territories with a single food cache, whereas squirrels use multiple storage places located in larger territories. Indeed, chipmunks showed lower basal proliferation rate than did squirrels but, interestingly, not a lower number of immature neurons. A possible explanation of these findings is the enhanced turnover rate of the newly generated cells in the squirrels in comparison to chipmunks.

Likewise, comparison of Russian rodents having different home ranges indicated higher proliferation activity in wood mice, known to patrol large territories, in comparison to bank voles, which patrol smaller ones (Kikkawa 1964; Niethammer and Krapp 1978, 1982; Dell'Omo and Shore 1996; Amrein et al. 2004b). This difference was also reflected in automated home-cage learning tasks in which wood mice showed (moderately) better reversal of place-preference learning (Galsworthy et al. 2004). However,

Amrein et al. (2004a,b) also demonstrated a balance between proliferation activity and survival of newly generated cells. Yellow-necked wood mice showed an almost excessive daily proliferation rate of 1.1% of the resident granule cell population (**Fig. 1a,b**) that was correlated with an equally high proportion of apoptotic cells. In old wood mice, the reduced proliferation activity was compensated by reduced apoptosis, entailing an increased survival of the newly generated cells. In bank voles and European pine voles, the daily cell proliferation accounts for about 0.3% of the resident granule cell population, despite significant differences in total dividing cells, total granule cell numbers, and habitat type (Amrein et al. 2004a,b). Thus, the neurogenesis-dependent functionality of the hippocampus in relation to lifestyle and complexity of habitats in a sample of related species can only be inferred when taking into account the total number of granule cells in the dentate gyrus and, when possible, seasonal variations.

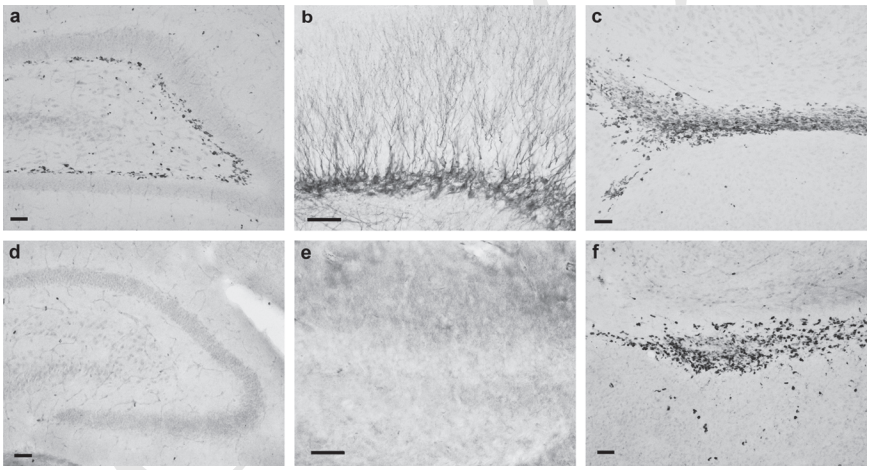

Figure 1. Two extremes in proliferation activity and neuronal differentiation. Adult yellow-necked wood mice (*Apodemus flavicollis; a,b,c*) show extreme proliferation activity in the dentate gyrus (*a*), where dividing cells are visualized with immunohistochemistry against Ki-67. Many of these cells in the subgranular layer (SGL) differentiate into neurons, as visualized with Doublecortin immunohistochemistry (*b*). Proliferation and migration of cells can also be seen in the rostral migratory stream (RMS), where Ki-67-positive cells line up and travel to the olfactory bulb (OB) (*c*). In adult short-tailed bats (*Carollia perspicillata; d,e,f*), a few Ki-67-positive, proliferating cells can be seen in the hilus, but no dividing cells are found in the subgranular layer of the dentate gyrus (*d*). Accordingly, no ongoing neuronal differentiation (doublecortin) can be found in the dentate gyrus (*e*). However, proliferation activity in the RMS (*f*) is similar to that in yellow-necked wood mice. Granule cell layer (GCL). Bar, 50 μm.

Most bat species thus far do not fit the picture of adult hippocampal neurogenesis thought to be essential for spatial cognitive abilities. Most bats lack neurogenesis in the hippocampus (Fig. 1d,e), despite their obvious need for navigational ability in territories far greater than the size of those found in similar-sized rodents. Whether this reflects another functionality of the bat hippocampus or less-investigated general functions of the hippocampus such as cognitive flexibility (not prominent in bats) remains an open question. In any case, the sole emphasis on spatial navigation as neurogenesis-dependent behavior may not be warranted. In fact, studies in laboratory rats that used irradiation to ablate new neurons in adults show no deficit in spatial navigation, even though long-term spatial memory was impaired. Spatial, episodic, and contextual memory, rather than spatial navigation, may be more dependent on neurogenesis (**see Chapter 21 by Abrous and Wojtowicz in this book**). This may be particularly important for predated rodents that must constantly relate danger to changing locations and stimuli, thus creating a particularly high demand for neurogenesis. The scarce adult neurogenesis of bats might also be linked to their astonishing longevity (Wilkinson and South 2002), as age is the most common down-regulator of mammalian adult neurogenesis.

WHAT IS REQUIRED TO STUDY ADULT NEUROGENESIS IN WILD-LIVING POPULATIONS?

Comparisons of neurogenesis across species should take age of the animals into account. Age may dictate not only rates of cell proliferation, which are clearly age-dependent, but also rates of differentiation and maturation.

Methods that minimize confounds due to handling stress and permit standardization across species should be used. The use of BrdU as a proliferation marker is impractical for the study of wild-living populations. Its popularity in rodent studies is due to the fact that its incorporation into the DNA permits one to follow the fate of the labeled cells. However, reliable labeling, quantification, and follow-up of a representative pool of dividing cells can only be achieved if the animals can be kept in captivity. Allowing for an approximate cell cycle time of 24 hours in rodents and perhaps longer in other species would require holding captured animals for days. This creates stress at the time of incorporation of BrdU that may influence the rate of neurogenesis. For example, stressed marmoset monkeys show a distinctly lower number of BrdU-labeled cells in the dentate gyrus, even with a postinjection survival time of only 2 hours (Gould et al. 1998). Furthermore, dosage, uptake, toxicity, and penetration

of BrdU or other thymidine analogs through the blood-brain barrier would have to be tested in each species in advance, substantially increasing the number of animals to be trapped and sacrificed and excessively inflating the costs in terms of manpower and infrastructure.

Recent advances in immunohistochemical labeling clearly show that the use of BrdU is no longer a methodological necessity. It has been shown that the number of cells labeled by Ki-67 in the rat dentate gyrus correlates closely with the number of cells revealed by BrdU injections (Kee et al. 2002; Wojtowicz and Kee 2006). Other studies have proven the reliability of endogenous markers versus thymidine analogs for identifying proliferating cells and young migrating or differentiating neurons (Tye 1999; Rao and Shetty 2004). In particular, Ki-67 and MCMs (replication-initiating factors) are evolutionarily highly conserved proteins that can be visualized across a wide range of species from different phylogenetic families. Thus, a simple combination of available markers for different stages of replication, glial/neuronal differentiation, and developmental stages of neurons, including apoptosis, would be sufficient for a detailed comparative analysis without having to introduce unnecessary confounds of the BrdU technique. Finally, comparative data contributing to any functional conclusion or theory about the role of newly formed granule cells in the mammalian hippocampus need some form of standardization. Finding 1000 proliferating cells in a dentate gyrus comprising 500,000 granule cells implies an other functionality than finding the same number in a larger-sized hippocampus containing 10 million granule cells. Up to now, there have been large methodological differences in referencing proliferating cells to the remaining dentate gyrus—if this is done at all. Data have been presented as density measurements relative to area or volume, as ratio to the total number of resident granule cells, or as a fraction of mature granule cells. We suggest that the preferred requirement for species comparison of proliferation activity should be to the area of the subgranular zone, whereas neurogenesis and cell turnover should ideally be standardized to an estimate of the total granule cell number.

The value of these methods notwithstanding, an ideal study of adult neurogenesis in a natural population would include BrdU. Many of the species that could be studied live in restricted areas that can be surveyed by dedicated neuroscientists. Animals can be trapped, injected with BrdU, tagged, fitted with telemetry transmitters, and retrapped again. Seasonal variation can be taken into account and appropriate numbers of animals can be killed and processed for BrdU immunohistochemistry in combination with phenotypic markers. Even more challenging, but still feasible, are studies that would involve reduction of neurogenesis by irradiation,

for example, and rereleasing the animals into their natural environment. This experiment would provide an ultimate test of the adaptive significance of neurogenesis.

CONCLUSIONS AND FUTURE DIRECTIONS

Despite the paucity of studies on adult hippocampal neurogenesis in wild-living populations, some conclusions can already be drawn:

1. Across mammalian orders, such as primates, rodents, and bats, there are enormous species differences in terms of basal proliferation and survival of newly generated granule cells that cannot yet be explained by cognitive ability and behavioral specializations.
2. Neurogenesis is age-dependent in all species examined thus far.
3. The species-specific levels of adult hippocampal neurogenesis may also depend on other species-specific cytoarchitectonic characteristics of the hippocampus. For example, a relative size of the dentate gyrus to the rest of the hippocampal formation may indicate how important the classical trisynaptic pathway is in comparison to alternative afferent inputs into the hippocampus (**Abrous and Wojtowicz, Chapter 21 in this book**). Size variations of the infrapyramidal mossy fiber (IIP-MF) projections in the hippocampus of rodents, i.e., of the axons of the dentate granule cells, should also be considered (for review, see Lipp 2007).

SUMMARY

There is an increasing awareness shown in the literature that if we are to make headway in our understanding of adult neurogenesis and of its significance, a broad range of species should be examined (see, e.g., Boonstra et al. 2001; Nottebohm 2002; Lindsey and Tropepe 2006). We suggest that at least three approaches would be profitable.

First, undertake detailed observational studies on each of a broad range of species from the natural world, particularly rodents, as this is the group that has been the major focus in laboratory studies. Such studies integrating detailed knowledge of animal's ecology, behavior, physiology, and evolutionary relatedness will help us to understand how season, sex, and environmental variables change rates of production and survival of new neurons.

Second, carry out experimental manipulations on these species to discriminate cause from effect in real-world scenarios (e.g., manipulating

stressors such as conspecifics or predators) or semireal-world settings. Such studies should be intraspecific, comparing the same species but from areas where the environmental needs may select for different rates of production and survival.

Third, carry out interspecies studies, both of closely related species with markedly different life histories and of distantly related species. Research on laboratory animals provides useful conceptual and methodological guidelines that can now be employed in studies of natural populations.

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