Consequences of Distant Hybridization in Animals

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By

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The book is dedicated to Prof. Eugeniy Panov for his outstanding contribution to the studies of ethology and evolution.

"...Everything that we achieve by art can and is carried out thousands and thousands of times by nature, and thus accidental and voluntary mixtures between animals are often obtained..."
—J. Buffon. Natural History of Birds, 1771

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PREFACE

Hybrids and their genomes have served as a fascinating model for genetics, even before the discovery of DNA. When two evolutionarily distinct genomes are combined in a hybrid zygote through interspecies hybridization, they experience dramatic regulatory and epigenetic reprogramming, often referred to as a "genomic shock." During this process, the structural and coding differences of the parental genomes are reconciled, sometimes giving rise to novel genetic features. These genetic innovations can offer valuable insights into how new phenotypes, and occasionally even new species, originate, adapt, and evolve. It is within this rich and complex field of inquiry that Prof. Michael Evgen'ev has made groundbreaking contributions throughout his five-decade-long career. His research, which began with his first publication in 1969, spans studies on animal hybridization, transposable elements, and the heat shock response. These interconnected fields are skillfully explored in *Consequences of Distant Hybridization in Animals*, co-authored with his longtime collaborator, Dr. Sergei Funikov, from the Engelhardt Institute of Molecular Biology (EIMB) in Moscow.

The book is divided into several parts, each delving into critical aspects of hybridization and molecular biology. Part 1 explores the ecological and ethological mechanisms that prevent hybridization, as well as the formation of hybrid zones and the genetic consequences of interspecies hybridization across various taxa. It begins with an overview of isolating mechanisms that prevent hybridization, drawing heavily on Ernst Mayr's hierarchical system of isolating mechanisms. These mechanisms include ecological, ethological, mechanical, and genetic barriers that reduce the chances of hybridization in nature. For instance, many species are ecologically isolated by their habitats or breeding periods, and certain species have evolved specific behaviors that prevent interbreeding with closely related species. However, under some conditions, such as ecological disturbances, these barriers may break down, leading to hybrid zones where hybridization can occur between species that usually do not interbreed. These zones are dynamic and subject to various outcomes, such as reinforcement of species boundaries or the formation of new species through hybridization. The authors also discuss the prevalence of interspecific hybridization in various animal groups, noting that hybridization seems more frequent in taxa like birds, where reproductive barriers may be relatively less rigid.

Part 2 shifts the focus to the molecular organization of the eukaryotic genome, emphasizing chromatin structure, epigenetics, and the critical role of small non-coding RNAs (miRNAs, piRNAs) in genome stability. The authors' seminal studies on transposable elements (TEs), such as *Penelope* and *Ulysses*, are highlighted here, providing readers a glimpse into the evolutionary significance of these mobile genetic elements. Their work on transposable element mobilization across species borders, particularly in the context of hybrid dysgenesis, has been crucial in demonstrating the dynamic and often disruptive role TEs play in genome evolution. Through meticulous genetic crosses, Professor Evgen'ev and his team introduced chromosomes from one species of the *virilis* group into the genome of another, leading to the discovery of multiple "jumps" of transposable elements within hybrid genomes. This work revealed the fluidity of the genome and its evolutionary consequences. Among the four transposable elements he described—*pdv*, *TV1*, *Ulysses*, and *Penelope*—*Penelope* stands out as particularly significant. Collaborating with scientists like Irina Arkhipova and Matthew Meselson from Harvard, the team identified *Penelope* as the founding member of what is now known as the Penelope-like elements (PLEs) superfamily, found across hundreds of species. Some researchers even suggest that PLEs constitute a distinct class of transposable elements. This part of the book also delves into the mechanisms by which small RNAs, particularly piRNAs, silence transposable elements and ensure genome integrity, an area where the authors have made significant contributions.

Part 3 focuses on the cytogenetic and molecular aspects of hybridization, a continuation of the authors' long-standing interest in how hybridization influences genetic recombination and genome stability. In parallel with Evgen'ev's pioneering work on TEs, he and his associate Elena Lozovskaya described a new form of hybrid dysgenesis in *Drosophila virilis*, which advanced our understanding of the genetic mechanisms underlying hybrid sterility and transposable element activation. Unlike the well-known P-M and I-R syndromes in *Drosophila melanogaster*, the dysgenic crosses in *D. virilis* led to the mobilization of multiple TEs, with *Penelope* probably playing a key role. This discovery deepened our understanding of how hybridization can drive both genetic instability and evolutionary innovation.

Part 3 also reflects Evgen'ev's early work on Drosophila hybrids, where he demonstrated how polytene chromosome pairing patterns in salivary glands correspond to recombination processes in meiosis. These foundational insights into genetic stability in hybrids stem from his initial discovery of heterozygous inversions in *Drosophila funebris*, which laid the groundwork for further studies on sympatric speciation. By leveraging hybrids between species in the *virilis* group of *Drosophila*, he demonstrated that the patterns of polytene chromosome conjugation mirrored meiosis pairing patterns, a key process in recombination. His discovery that asynaptic regions in polytene chromosomes do not participate in recombination added a new dimension to our understanding of genetic inheritance and speciation.

The authors' research has not only advanced our understanding of the molecular mechanisms governing hybridization and genome stability but also challenged traditional views on species boundaries and evolutionary processes. Their extensive work on heat shock proteins, detailed in other publications, complements the research presented here, reinforcing the broader theme of how organisms adapt to genetic and environmental stressors.

In particular, this book represents the culmination of Prof. Evgen'ev's lifelong dedication to unraveling the complexities of inheritance, hybridization, and genome evolution. It stands as a testament to his lasting impact on the fields of molecular biology and evolutionary genetics. As we continue to explore the profound implications of hybridization in nature and recognize its far greater prevalence across species (including our own!), his work will remain an essential reference for future generations of researchers.

It is a great honor to write this preface and introduce the remarkable work of Evgen'ev and Funikov. Their book encapsulates decades of groundbreaking research and critical contributions to the fields of molecular biology and evolutionary genetics. By exploring the intricate processes of inheritance, hybridization, and genome evolution, they have significantly shaped our understanding of how genetic diversity and evolutionary change occur. This work not only reflects their profound insights but also serves as a vital resource that will continue to inspire and guide new research efforts in these ever-evolving fields.

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INTRODUCTION

It is now beyond doubt that interspecific hybridization is a widespread phenomenon found in both animal and plant kingdoms. The important role of hybridization in the evolution of wildlife and the source of the emergence of new species and life forms was noted by the great taxonomist and naturalist Carl Linnaeus (Linnaeus 1751). Linnaeus obtained his conclusions concerning the role of hybridization in speciation by crossing plants. In his pioneering work, he divided species into "primary" species, i.e., those resulting from creation, and "secondary" species, that can arise from hybridization of the primary forms. Interestingly, Linnaeus was the first to notice that interspecific hybrids are more common in plants than in animals. This conclusion was further confirmed by numerous researchers from different countries, including Darwin himself (Darwin 1877). It should be noted that the term "hybridization" is rather general and is used differently by different authors. Initially, the term 'hybridization' began to refer to the crossing of animals or plants belonging to different species or even genera. The offspring of such hybridization are called 'hybrids' using the term of Greek origin 'hybrid'. Such hybridization is usually called 'distant hybridization'. Quite often the resulting F1 hybrid offspring is distinguished by "greater hybrid vigour", i.e. the phenomenon of heterosis, first described by Darwin, who devoted a chapter to distant hybridization ("hybridism") in his great book (Darwin 1859). It should be noted that almost the whole of this chapter has been based mainly on the results of plant crossing experiments carried out by Joseph Kölreuter (1733-1806) and Karl Gärtner (1772–1850) while Darwin's account of animal hybridization was scanty (citation from excellent review by (Borkin and Litvinchuk 2013)), although already in the 19th century a few studies on animal hybrids appeared (Morton 1847; Watson and Wailly 1893).

Darwin's Origin of Species is often criticized for not paying enough attention to the mechanisms of speciation. Indeed, it was difficult for Darwin in his time to discuss the causes of sterility and low viability often observed in interspecific hybrids (Presgraves 2010). On the other hand, in the chapter discussing interspecific hybridization, Darwin came surprisingly close to understanding the causes of sterility and non-viability often observed in distant hybrids. Thus Darwin, with no knowledge of the laws of genetics, formulated the fundamental ideas about interspecific hybrids. He drew conclusions that were quite revolutionary in his time. Darwin, as well as many of his followers and opponents (Bateson 1909; Huxley 1893), rightly believed that the frequently observed low viability and sterility of interspecific hybrids represented one of the greatest unsolved problems of evolutionary biology. Thus, Bateson wrote that until the problem of hybrid sterility is solved, we will have no acceptable account of the origin of species. Based on the very limited information available at the time, Darwin concluded that the sterility often observed in interspecific hybrids was not endowed by a creator nor directly favoured by natural selection but rather evolved as incidental by-products of interspecific divergence (Darwin 1859). In other words, the sterility of species hybrids is observed when their development is "disturbed by two organizations having been compounded into one." In addition, Darwin was the first to observe that the level of sterility is directly proportional to the phylogenetic distance between the crossed species, with hybrid males being more likely to be sterile than females. Much later, with the development of genetics, other researchers (Bateson 1909; Dobzhansky 1982; Mayr 1982; Mayr and Huxley 1954; Muller 1942) put Mendelian details to Darwin's inference that the species-specific factors (i.e. genes) controlling development may be sometimes incompatible and formulated "biological concept" of species. It is the various causes of such incompatibility and the resulting sterility often observed in hybrid organisms that this paper is essentially devoted to.

It should be noted that hybridization is sometimes used to call crossbreeding between lines or even populations of the same species that differ in the content of several genetically determined traits. Hybridization is also usually divided into natural hybridization, which occurs in nature, and artificial hybridization, which is carried out by humans to develop new breeds of animals or plant lines (Serebrovski 1935). Darwin himself noted that with the domestication of animals and artificial hybridization, people manage, for obvious reasons, to obtain hybrids that cannot arise in nature.

The first experiments on artificial interspecific hybridization were carried out using plant species and took place in Germany, England and Russia in the late 18th and early 19^{th} centuries e.g. Joseph Koelreuter (1733-1806; Carl Gartner (1772-1850); Charles Naudin 1815-1899 (Quote from (Gaisinovich 1988)). Later, by crossing peas, Gregor Mendel laid the foundations of genetics as an exact science (Mendel 1866). It should be noted, however, that the regularities described by Mendel are often violated in the most unexpected way during distant hybridization. Thus, the result of distant hybridization, in addition to the very common complete or partial sterility of F1 hybrids, can be non-disjunction or elimination of chromosomes of one of the parental species, as well as a variety of disorders in gene expression and behaviour of chromosomes in mitosis and meiosis of hybrid forms.

The long-standing dispute over the term "hybridization" itself and its application to different crosses continues to this day. Thus Charles Naudin as early as 1815 generally believed (quote from (Gaisinovich 1988) that there is no fundamental difference between species, races and varieties, and the British researcher Lowe, studying different species of birds, put forward his own concept explaining all the diversity of birds in nature as a result of interspecific hybridization (Lowe 1930, 1936). In particular, this researcher explained by interspecific hybridization the remarkable diversity of some groups of birds on the islands, for example, the diversity of extinct moas of New Zealand (38 species in 5 genera) or Galapagos finches (22 species). The Dutch botanist Jan Lotsy (1867-1931), who proposed the concept of evolution through hybridization of species, came to similar conclusions in the early 20th century. On the other hand, Linnaeus' famous antagonist Georges Buffon, who also paid much attention to the problem of hybridization, came to opposite conclusions. Discussing this phenomenon in animals, he proposed the so-called "sexual method" as a criterion of species. In other words, the absence of offspring or sterility of F1 hybrids from his point of view unambiguously indicated that the crossed forms belonged to different species, while the fertility of hybrids indicated that the crossed forms belonged to the same species.

Interestingly, Buffon was also the first to notice that birds are more prone to interspecific crosses than mammals, which was confirmed later by numerous authors (Panov 1989, 2001, 2005). The importance of interspecific hybridization in evolution was also disputed by the famous Russian scientist Peter Pallas (1741-1811), who, speaking about animal variability, rejected Linnaeus's ideas about the important role of interspecific hybridization, and even identified the main reasons that, from his point of view, prevented interbreeding between species in nature (Pallas 1780). Interestingly, the ideas of Buffon and Pallas found their numerous supporters in the last century in the formation of the "biological" concept of species, substantiated by the works of several brilliant geneticists (Dobzhansky 1982; Mayr 1940, 1947, 1982; Muller 1942; Heinisch 1964), which is still accepted by many scientists. According to this "biological" concept, developed in detail within the framework of the synthetic theory of evolution in the 20th century by the above-mentioned geneticists, a species is a genetically closed system with many isolating mechanisms developed in the course of divergent evolution. The proponents of the biological concept have come to assert that the criterion of morpho-biological uniqueness of a taxon, which was previously considered to be the main criterion, is purely subordinate to the criterion of reproductive isolation. According to the founder of this concept, Ernst Mayr (Mayr 1947, 1982), “species are defined not by differences but by separateness”. These authors and their followers generally considered and still consider interspecific hybridization between animals as a rare phenomenon or even an anomaly. According to these ideas, the rare cases of interspecific crosses with partially or completely sterile offspring, sometimes observed in nature, are necessary to select for greater efficiency of the isolating mechanisms already existing in the species.

It should be noted that in parallel with the biological concept of species, there are several other views on species and speciation in the scientific literature, that are not inferior to it. A common feature of these views is that the criteria for species are less rigid and not as categorical as in the biological concept (e.g. (Anderson 1953; Arnold and Meyer 2006; Barton 2001; Panov 1989)). In any case, the criterion of reproductive isolation is not decisive in any of them, which in itself, from our point of view, allows to avoid many terminological difficulties and inconsistencies that the biological concept constantly encounters in the question of species boundaries. These are the belief systems called “phylogenetic”, “evolutionary”, and “zoogeographic” concepts of species. Their comparative analysis is given, for example, in the reviews by Haffer (Haffer 1982) and Panov (Panov 1989).

At present, the great importance of distant hybridization in evolution and speciation in different groups of animals and plants is recognized by many field botanists and zoologists. Thus, Mallet suggested that about 10% of animal species, mostly younger ones, hybridize and continue to exchange genetic material at present (Mallet 2005). Moreover, interspecific hybridization is considered by many researchers as an important factor of evolution, providing adaptation of both plants and animals to changing environmental conditions and, in some cases, as a source of formation of new forms. Indeed, as it has become clear in recent decades, interspecific hybridization, widespread among many organisms, can lead to very different results. For example, hybrids can interbreed with one or both parental species, increasing their genetic diversity or, under some conditions, even giving rise to a new species. The diversity of results of distant hybridization has one thing in common: they are unpredictable and their causes are usually unknown. It is interesting to note that it was not until about the 1920s that the first results of studies of distant hybridization of animals in nature began to appear systematically (Haldane 1922; Hovanitz 1943; Lonnberg 1905, 1929; Serebrovski 1929; Phillips 1915). It is thanks to such population studies that the ideas of numerous supporters of the biological concept of species about various isolation mechanisms, which, in their opinion, practically prohibit interspecific interbreeding of animals in nature, dominating for many years, have been questioned. It should be said, however, that as a result of many years of research, both field biologists and laboratory scientists, based on the study of a wide variety of animals, have indeed described an elegant system of isolation mechanisms preventing at different levels the interbreeding between representatives of different species and higher taxa.

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PART 1:

ECOLOGICAL ASPECTS OF ANIMAL HYBRIDIZATION

CHAPTER ONE

ECOLOGICAL AND ETHOLOGICAL ISOLATING MECHANISMS PREVENTING DISTANT HYBRIDIZATION

Ernst Mayr, a well-known geneticist and ornithologist, one of the main proponents of the biological concept of species, formulated a clear hierarchical system of isolating mechanisms preventing interspecific interbreeding as early as 1947 (Mayr 1947, 1968). Mayr divides these mechanisms into ecological, ethological, mechanical and genetic.

Figure 1-1. Ernst Mayr and Michael Evgen'ev (translator) at the Koltzov Institute of Developmental Biology, Moscow, USSR, 1969.

Ecological factors may prevent potentially interbreeding species from meeting for various reasons. Ecological isolation is usually very effective. Thus, it has long been known that many well-studied closely related species occupy different habitats during the breeding season or begin breeding at different times (Andersen et al. 2023; Hovanitz 1943; Mayr 1963; Mendelson and Safran 2021; Panov 1989; Snow 1954; Spieth 1958; Panov 1968). For example, phylogenetically similar Drosophila species *Drosophila pseudoobscura* and *Drosophila persimilis*, two classic species in speciation research, are easily crossed in the laboratory but do not form hybrids in nature because they occupy different habitats and fly at different hours of the day (Carson 1951; Fan et al. 2013; Nanda and Singh 2012). Recent genome assemblies have revealed fixed differences in inversion and transposable element content between these closely related Drosophila species (Carpinteyro-Ponce and Machado 2024). Spatial and temporal isolation are not mutually exclusive. Disturbance of spatial isolation of closely related species, often with anthropogenic causes, e.g. due to plant associations change, as well as changes in the precise timing of reproduction of closely related species, may create prerequisites for hybridization.

In the case of ecological isolation violation occurring for various reasons, the first place is occupied by fixed, practically independent of external factors, isolation mechanisms, that are based on the genetically determined phenomenon of selective mating with a partner belonging to the same species. Sometimes mating between close species is impossible due to mechanical reasons, namely differences in the structure of copulatory organs. Selective mating is also based on genetically fixed species-specific behaviour of partners and is the subject of actively developing ethology. The Austrian scientist Lorenz played a major role in the formation of ethology as a science with a developed methodology. He received the Nobel Prize together with Karl von Frisch and Nikolaas Tinbergen in 1973 “for discoveries related to the creation and establishment of models of individual and group behaviour of animals”.

Whereas before Lorenz and Tinbergen, scientists had studied mainly the influence of external factors on animal behaviour under artificially created conditions, these researchers shifted their focus to the influence of internal, hereditary factors on animal behaviour under the conditions of their natural environment (Lorenz 1939; Tinbergen 1952). They described highly complex behaviours that could not be acquired by learning and are therefore genetically programmed. Thus, the founders of ethology proved that animal behaviour is highly determined by genetics and, therefore, must be subject to natural selection and other evolutionary and genetic factors such as point and chromosomal mutations and gene drift.

Numerous studies have shown that during the breeding season, individuals belonging to one species actively seek each other out, while individuals of other closely related species are generally rejected. The signals by which individuals of one species recognize individuals of another can be of very different nature. Apparently, the most common system for recognizing individuals of one's own species is signalling using attractants as well as other chemicals (Gilbert 1963; Gilbert and Thompson 1968; Kidyoo et al. 2022; Li et al. 2023; Mayr 1950; Queffelec et al. 2023; Panov 1968). Signalling by specific odorants is widespread at all levels of animal community organization, from rotifers to flies and mammals. An example of such molecular substances providing reproductive isolation and intraspecific mating is described in Drosophila and in many insects, a major driver of the isolation is represented by cuticular hydrocarbon pheromones (Mas and Jallon 2005). These pheromones help to identify potential intraspecific mates. When the distributions of related species overlap, there may be strong selection on mate choice for intraspecific partners because interspecific hybridization usually results in sterility and significant fitness costs.

While *Drosophila melanogaster* for many years was a key model for the investigation of reproductive isolation, the genes underlying species recognition remained largely unknown. The molecular mechanisms underlying Drosophila speciation and sexual recognition were explored by measuring a tissue-specific cis-regulatory divergence using RNA sequencing in *Drosophila simulans* × *Drosophila sechellia* hybrids. The investigation focused on cis-regulatory changes specific to female oenocytes, the tissue that produces cuticular hydrocarbons. The analysis performed enabled the identification of a small number of candidate genes. Interestingly, one of these genes, the fatty acid elongase *eloF*, strongly affects the hydrocarbon levels present in *D. sechellia* and *D. melanogaster* females, as well as the propensity of *D. simulans* males to mate with them. It was concluded that pronounced cis-regulatory changes in the transcription of *eloF* may be a major driver in the sexual isolation of *D. simulans* from multiple other species of Drosophila including siblings such as *D. melano*gaster (Combs et al. 2018).

Another remarkable example where a mutation in just one gene can lead to reproductive isolation and sympatric speciation was obtained in a pair of other Drosophila close species (Ortíz-Barrientos and Noor 2005). Several theoretical models have demonstrated that speciation due to certain gene flow can occur readily via a “one-allele mechanism”. According to this model, the spread of the same allele within both of two diverging species reduces their subsequent hybridization. Such an allele has been described in

D. pseudoobscura. Interestingly, the described alleles conferring low or high assortative mating in *D. pseudoobscura* produce the same effects when inserted into the genome of another close species (*D. persimilis*). These experiments suggest that the type of genetic variation that is most conducive to several rather controversial modes of speciation with gene flow, such as sympatric speciation, is present in nature. Other examples of genetic systems that provide molecular-level selectivity for hybridization within a species, as well as examples of violations of such prohibitions, will be discussed in the second part of this book.

The Drosophila species by no means represent the only example of how a single gene controlling behaviour can lead to sterility in distant crosses. For example, it was shown that two sympatric strains of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae), termed corn strain (C) and rice strain (R), referring to their most common host plants, were reproductively isolated. Thus, when R females mate with C males, the resulting RC hybrid females exhibit dramatically reduced fertility independent of their mating partner. It was also shown that the reduced fertility in this case is caused by the fact that these females are behaviorally sterile i.e. refrain from mating. Moreover, a Z-chromosomally linked sterility locus was experimentally localized and it is probably incompatible with a few autosomal factors, leading to the observed sexual abstinence (Kost et al. 2016).

There is other evidence in the literature that isolation between species may depend on just one genetic factor (Ortíz-Barrientos and Noor 2005). Sound signalling or singing by males is also very common among animals, playing a major role in recognizing individuals of their species in birds, some insect species, mammals, and even fish and crabs (Wynne-Edwards 1962; Gottsberger and Mayer 2019; Nevo et al. 1987; Seddon 2005; Wilkins, Seddon, and Safran 2013).

In most different groups of animals from fish to birds and mammals, species-specific features of colouration and body shape perceived by other individuals of the species with the help of visual analyzers play an important role. A more detailed consideration of the ecological and ethological isolation mechanisms that prevent interbreeding between different species is beyond the scope of this book, as there are numerous excellent reviews devoted to this problem (Johansson and Jones 2007; Merrill et al. 2024; Spiegel et al. 2016).

In our review, we will focus on the consequences of hybridization, which has already occurred despite the existence of very effective isolating mechanisms that usually hinder interspecific crosses. Indeed, it turned out that despite the existence of all these isolating mechanisms, interspecific crosses in nature occur quite frequently. In particular, extensive population studies of areas of secondary contact between originally allopatric species and subspecies of different groups of animals especially birds have played an important role in the detection of interspecific hybrids. Such contacts are sometimes of anthropogenic origin and may have occurred relatively recently.

Ernst Mayr and other proponents of the biological concept of species put forward as one of its main postulates the idea of strengthening reproductive isolation in secondary contact zones of diverging related species by selection against F1 hybrids, which often exhibit sterility and are inferior in many adaptive traits to the parental forms (Bigelow 1965; Mayr 1982; Sibley 1961; Mayr 1963). Although proponents of this concept admit rare cases of "merging" of species, they consider them as obvious anomalies, owed mainly or exclusively to anthropogenic factors. However, intensive study of secondary contact zones between species and subspecies has shown that interspecific hybridization in nature, although with different frequencies, did occur and still occurs in different groups of animals and plants. Moreover, the strengthening of reproductive isolation between crossing species in the hybrid zone is by no means a general rule. Interesting results in favour of this conclusion were obtained in genetic experiments using multiple-choice experiments (Anderson and Kim 2005) exploring two close species of Drosophila. To test whether, as postulated by reinforcement theory, sexual isolation between species in sympatry is strengthened by natural selection against maladaptive interspecies hybrids, closely related Drosophila species with overlapped distribution were used. Thus, *D. pseudoobscura* and *D. persimilis* from four locations where these species are sympatric, and from three locations where only *D. pseudoobscura* has been found, were utilized in studies of sexual isolation. A multiple-choice technique was used to record matings between sympatric and allopatric strains of the two species. The performed experiments have demonstrated that the average isolation index for sympatric strains of these species was not significantly different from the average index estimated for allopatric strains.

On the other hand, the emergence of hybrid populations in the zone of secondary contact between closely related forms has proven to be a very common phenomenon in a wide variety of animals. In particular, distant hybridization in birds and its evolutionary consequences was described and discussed in the middle of the last century by the famous German ornithologist Erwin Stresemann, and later by many other researchers (de Raad et al. 2022; Panov 1989, 2001; Stresemann 1951; Stresemann et al. 1975).

The widespread interspecific hybridization among animals, discovered in nature in recent decades due to the development of molecular biology methods, has inevitably led to a reassessment of the importance of distant hybridization as a factor of evolution, as well as to a new, deeper understanding of the problem of species and speciation. It should be said that the terms "hybridization" and "distant hybridization" itself, in particular, have been used differently by different researchers. Thus, many researchers, like Prof. Serebrovski, who devoted much time to the study of this phenomenon (Serebrovski 1935), used the term "hybridization" to designate crosses between individuals belonging to different species. This definition only partially coincides with the wording adopted in the International Code of Zoological Nomenclature (Nomenclature and Sciences 1999) according to which a hybrid is a descendant of two individuals belonging to different taxa.

Thus, Serebrovski in his book devoted to the hybridization of animals, leaving the terms "species hybridization" and "hybrids" for the area of interspecific crosses. Serebrovski (Serebrovski 1935) suggested crosses at the intraspecific level (subspecies, races, breeds) should be called subhybridization (subhybrids) and hybridization between members of different families and higher taxons - ultrahybridization (ultrahybrids). He suggested the latter term, for example, for experiments on the hybridization of sea urchins with molluscs, but this terminology did not take root in the scientific literature. On the other hand, many evolutionists have rightly noted that when considering various crosses, it is difficult to divide them into intraspecific, interspecific, and intergeneric ones based on the characteristics of the resulting hybrids.

In this book, following many researchers, we use the term "hybridization" (Harrison and Larson 2014; Hodges, Burke, and Arnold 1996; Woodruff and Gould 1987) to refer to both interspecific crosses and crosses between individuals of two morphologically contrasting populations or even lines within the same species that are distinguishable by one or more heritable traits, e.g. content of transposable elements (TEs).

In this respect, we are close to the point of view of Prof. Panov (Panov 1989), who defined hybridization as the "crossing of individuals belonging to different populations, if there is a normal gap (hiatus) between such populations by some morphological traits, be it size characteristics, colouration features, or differences in karyotype structure". Similarly, according to Prof. Bigelow (Bigelow 1965), hybridization was defined as "crossbreeding between natural populations that have diverged sufficiently to exhibit the effects of genetic incompatibility recognized as such".

The complete or partial sterility of first-generation hybrids is often a manifestation of such incompatibility. At the same time, we are well aware of the fundamental differences between the results of distant (i.e., interspecific) crosses and the results of crosses between lines of the same species differing, say, in the content of TEs, as observed in "dysgenic" crosses between Drosophila lines of the same species. In the latter case, fully or partially sterile offspring also appear, described as the phenomenon of "hybrid dysgenesis" (Bingham, Kidwell, and Rubin 1982; Kidwell, Kidwell, and Sved 1977).

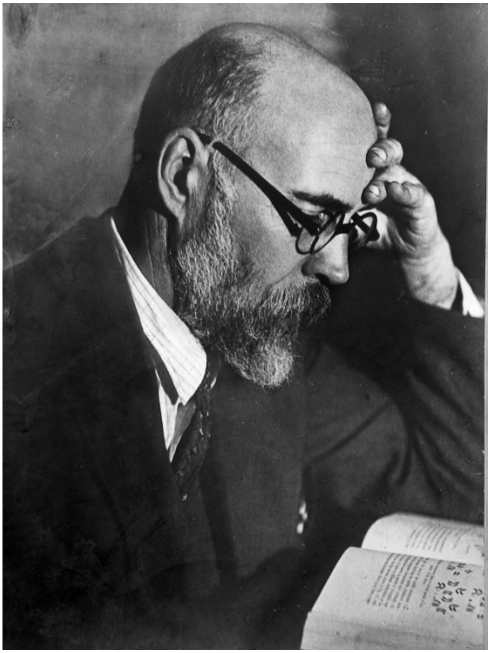

Figure 1-2. A famous Russian biologist. The author of a pioneering book about hybridization in animals. Alexander Serebrovski (1892-1948).

Serebrovski rightly pointed out that despite some conventionality in defining the boundaries of species as a systematic unit, denying the expediency of separating interspecific hybridization into a more or less specific area of research may lead to emasculating the whole concept of hybrids (Serebrovski 1935). On the other hand, the approach we use in this review, involving the analysis of both interspecific and interline hybrids, allows a deeper understanding of the consequences of hybridization *per se* at the genetic and molecular levels. This approach also allows us to understand the mechanisms underlying many of the phenomena described earlier when studying hybrids between members of different species or even higher-level taxa (genera, families, etc.). It is of note also that in our analysis we included only the cases of hybridization described in animals, leaving out numerous cases of hybridization occurring among plants and bacteria, as well as the phenomenon of horizontal gene transfer between species belonging to different taxa, which is widespread and is obviously of great importance in evolution and speciation.

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