Abundant empirical evidence of multilevel selection revealed by a bibliometric review

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Abstract

Natural selection is based on the notion of differential reproduction between entities, often characterized as a struggle between individual organisms. However, natural selection can act at all levels of biological organization, thus being termed 'multilevel selection' (MLS). A common misconception is that MLS lacks empirical support. To address this, we conducted a bibliometric review of 2,950 Web of Science/Scopus-indexed scientific articles. Our goal was documenting the range of taxa/systems, levels, and research topics/tools where MLS has been used to understand natural selection across levels. We found 280 studies providing empirical support for MLS: 100 were performed *in situ*, 180 were laboratory experiments. The studies span a vast range of organisms, from viruses to humans and eusocial insects. While 90.4% of studies focused on some form of organismal group (demes, colonies, aggregates), the remaining 9.6% explored selection at other levels (communities, cells, nuclei). We classified these 280 studies into research categories such as artificial selection, breeding through group selection, indirect/social genetic effects, and contextual analysis, among others. In contextual analysis studies, the strength of selection was comparable across levels. Contrary to common notions, there is solid empirical support for the utility and importance of MLS in explaining natural selection and evolution.

Keywords: animal and plant breeding; artificial selection; contextual analysis; epistasis; group selection; units of selection.

Introduction

Multilevel selection (MLS) occurs when natural selection simultaneously acts at two or more different levels in a nested biological hierarchy (Damuth & Heisler, 1988; Okasha, 2006; Wilson & Wilson, 2007; Marín, 2024). Specifically, MLS occurs when there is differential reproduction of groups in addition to reproduction of individual entities within them, or when the differential reproduction of individuals is based on their group composition or characteristics (like the social environment) (see key definitions in **Box 1**) (Goodnight et al., 1992). Goodnight et al. (1992) have defined MLS as "variation in the fitness of individuals that is due to both properties of the individuals and properties of the group or groups of which they are members". Goodnight et al. (1992) definition incorporates models that explicitly include differential extinction of entire groups (e.g., Levins, 1970), trait-group models (Wilson, 1975), and Wright's (1945) definition of interdemic selection (which does not require group extinction).

The MLS framework has been useful, even essential in studying the central dogma in molecular biology (Takeuchi & Kaneko, 2019), horizontal gene transfer in bacteria (Lee et al., 2022), multicellularity (Bozdag et al., 2023), cancer (Aktipis et al., 2015), disease/virus evolution (Blackstone et al., 2020), animal (Craig & Muir, 1996) and plant breeding (Zhu et al., 2019a), as well as economics (Wilson et al., 2020) and cultural institutions (Wilson et al., 2023). The clear value of an MLS approach, whether related to the selection (emergence) of particular traits or to the discovery of what affects fitness in a given system/organism, is its focus on identifying both the direction and strength of selection from multiple sources. Despite this, criticisms and skepticism persists among biologists (Eldakar & Wilson, 2011) – albeit anthropologists seem to favor an MLS framework, according to a survey by Yaworsky et al. (2015). Marín (2024) has identified three main arguments in favor of MLS: first, the term "unit of selection" (Suárez & Lloyd, 2023; Lloyd, 2024) has a polysemic nature, with at least three different meanings (interactors, replicators/reproducers/reconstitutors, and manifestors of accumulated adaptations). Second, the fact that biological entities as complex as an organism or a gene must -at least- have evolved from less complex entities (Okasha, 2006). And third, there is vast empirical evidence for this theory both in laboratory and natural populations. Sound literature reviews of such empirical evidence of MLS can be found in: Wilson and Sober (1994), Goodnight and Stevens (1997), Eldakar and Wilson (2011), Goodnight (2015), Marín (2015, 2016, 2024), and in Hertler et al. (2020). Despite these clear reviews and a diversity of empirical studies across a range of taxa, the misconception that MLS lacks empirical support persists (Harms et al., 2023). Here we address this misconception head on, by revealing an abundance (not a paucity) of examples of MLS in a diversity of taxa and biological systems, levels of biological organization, and type of research topics and tools.

In evolutionary biology, the evolution of altruism has been a main focus of MLS debates for decades, but altruism is just one trait that can evolve via MLS. On the one hand, the classic example of the evolution of altruism considers groups within which selfish individuals outcompete altruists, while groups with more altruists contribute more offspring to the next generation than groups comprised of more selfish individuals (Darwin, 1871; Wilson & Wilson, 2007). On the other hand, MLS also occurs when emergent group traits (e.g., social network structure, density, collective colony personality, among other descriptors) have significant effects on the reproductive success of a focal individual (Damuth & Heisler, 1988; Goodnight et al., 1992; Philson et al., 2025). Such effects of emergent or contextual traits have been amply demonstrated, for example in studies of epistasis (Burch et al., 2024) and indirect genetic effects (IGEs) (Linksvayer et al., 2009; Buttery et al., 2010; Bijma, 2014; Baud et al., 2021; Santostefano et al., 2025), and using techniques such as contextual analysis (Marín, 2016; Suárez & Lloyd, 2023; Lloyd, 2024; Philson et al., 2025).

We conducted a bibliometric review of the scientific literature to identify the breadth and depth of empirical evidence for the critical contribution of evaluating MLS across levels of biological organization. In addition, we also focused on phenotypic selection studies that use contextual analysis (Heisler & Damuth, 1987) to decompose the strength and direction of selection at different levels (individual organisms and groups of organisms). We then organized the literature on the basis of study systems (i.e., plants, animals, bacteria, etc.), levels of biological organization assessed (demes, communities, microbiomes, cells, etc.), and type of research (i.e. *in situ* studies, artificial selection experiments, breeding through group selection, etc.). The focus of this review is to provide an introduction, accounting, and organization of the vast empirical support of MLS and its utility to understand the natural world. In this review, "support" means only that levels of selection were explicitly measured, not that higher levels or "group" selection outweighed lower levels. Due to obvious publication bias towards positive results (i.e., demonstrating strong selection at several levels), we remained strict in our inclusion/exclusion criteria (see below). Despite this, there were some studies in which higher-level selection or group properties were shown not to be important in explaining focal individual fitness (Philson & Blumstein, 2023a b). Thus our bibliometric review also captured studies in which individual-level selection might be the main selective force, something perfectly consistent with MLS. In addition, while discussions of alternative and complementary frameworks (such as inclusive fitness theory) and mechanisms that partition variation within and between groups (e.g. conditional dispersal, kinship and kin groups) are of general interest (see Frank, 2025), the consideration of such topics are beyond the scope of this review.

Materials and Methods

Bibliometric analysis: search terms

The current review is classified as a 'bibliometric' analysis and not as a 'meta-analysis' because, with the exception of the regression coefficients of 25 studies focused on contextual analysis (**Box 1**), no actual data was extracted from the articles. Rather, this review aimed at compiling the empirical evidence for MLS *in situ* and in laboratory experiments by conducting a bibliometric analysis following the 'Preliminary guideline for reporting bibliometric reviews of the biomedical literature (BIBLIO)' (Montazeri et al., 2023). Please find in the **Supplementary Table 1** (**Zenodo:** https://doi.org/10.5281/zenodo.16633276), the BIBLIO complete checklist required in such preliminary guideline.

In January 2025, the following terms were searched in the Scopus (https://www.scopus.com/home.uri) database: "multilevel selection" across the whole article, and "group selection" in the Title, Abstract, and Keywords – because the latter was the term most commonly used before Damuth and Heisler (1988). The search spanned 1900 – 2024 and included articles and reviews only published in English, in journals indexed both in Web of Science and Scopus. In Scopus, the following areas were excluded from the search: dentistry; nursing; energy; chemical engineering; health professions; pharmacology, toxicology and pharmaceutics; business, management, and accounting; materials science; physics and astronomy; engineering; computer science; arts and humanities; mathematics; and medicine. All the remaining areas were included in the search. We also conducted an additional search in Google Scholar, with the same terms as in the Scopus search, to capture Web of Science/Scopus-indexed MLS empirical papers not discovered by the Scopus search due to differences in both search engines.

Bibliometric analysis: identification, screening, eligibility, and inclusion criteria

The bibliometric analysis had a total of four phases: identification, screening, eligibility, and inclusion (**Fig. 1**). In the identification phase, all duplicates were deleted, and in the screening phase, based on information contained within the abstracts, all articles not related to biology, cooperation, and social behavior in general, were excluded. For the eligibility phase, all non-empirical studies were excluded, again based on the content within the abstracts. These non-empirical studies included mathematical models, reviews, discussions, response articles, conceptual models, and opinion articles, among others. In the inclusion phase, the articles were read in their totality, and those articles indicating MLS or group selection as 'possible' or 'plausible' (but not surely) mechanism explaining the observed results or patterns, were also excluded. For example, among the articles excluded on this third phase is an article entitled: "Sex-ratio bias and possible group selection in the social spider *Anelosimus eximius*" published in *The American Naturalist* by Aviles (1986), because the author indicates that group selection *might* be the mechanism explaining her results but further research is needed. All articles employing the same type of argumentation or reasoning were also excluded.

Bibliometric analysis: classification

After the inclusion phase, articles were classified according to the type of study (*in situ* or laboratory); taxon or study system (viruses, bacteria, eusocial insects, humans, microbiomes, etc.); the level of biological organization which was the main focus of research (groups or demes of organisms, communities, colonies, nuclei, aggregates, selfish genetic elements, etc.); and the main topic (or sub-topic) or method to assess MLS in situ or in the lab. For the latter, we identified a total of 16 categories and 67 sub-categories of topics and sub-topics of MLS empirical research (Table 1). A general overview and specific details, as well as information about the exclusion/inclusion criteria of each category and sub-category, can be found in the **Supplementary Methods** (**Zenodo:** https://doi.org/10.5281/zenodo.16633276). The full list of MLS empirical articles, after the inclusion (third) phase, can be found in **Supplementary Data** (**Zenodo**: https://doi.org/10.5281/zenodo.16633276).

The MLS *in situ* studies included 10 categories (**Table 1**; further information can be found in **Supplementary Material; Zenodo:** https://doi.org/10.5281/zenodo.16633276), as follows:

• ***Cultural multilevel selection***: those that investigated MLS in the spread of cultural traits, and, for example, demonstrated that traits conferring a group-level advantage can spread via cultural group selection.

• ***Molecular sequencing***: those that implemented any sort of molecular sequencing to natural populations, using different tools, from single-nucleotide polymorphism analysis to genome-wide association studies.

• ***Indirect Genetic Effects/Social Genetic Effects (IGE/SGE)***: an IGE has been defined as the "effect of a gene in the genome of one individual on the phenotype of another individual" (Wade, 2025). IGE/SGE studies collect population and trait and/or loci data to assess the effects of interacting partners on a focal individual traits' and/or reproduction.

• ***Group heritability:*** these studies assessed group heritability as the 'tendency of offspring groups to resemble their parental groups with respect to group-level traits' (Okasha, 2003).

• ***Group effects***: these studies assessed the effects of group emergent properties (like networks of interactions or group structure) on focal individuals' trait variation and/or individual fitness.

• *Dataset analyses*: these studies analyzed historical or published data to infer MLS processes occurring in natural populations or communities.

• *Contextual analysis*: contextual analysis extends the commonly used methods to measure natural selection in natural populations (Lande & Arnold, 1983; Arnold & Wade, 1984) by including "contextual" or "emergent" traits, that is, traits measured on the group or neighborhood, in the multiple regression. In this way, relative fitness is a function of individual and group or emergent traits.

• *Colony selection:* these studies directly measure phenotypic variation at the whole-colony level, in eusocial insects.

• *Phylogenetics*: these studies implemented phylogenetic analyses either to assess selection at the species level or to explain the evolution of complexity/multicellularity across phylogenetic trees.

• *Field experiment*: these field studies assessed group effects on focal individuals' phenotypic variation and/or fitness.

The MLS experimental studies included six categories (**Table 1**; further information can be found in **Supplementary Material; Zenodo:** https://doi.org/10.5281/zenodo.16633276), as follows:

• *Lab experiments*: some lab experiments imposed group and individual selection regimes and compared responses to selection afterwards, some measured the molecular consequences of such treatments, others measured group effects on focal individuals' fitness, microbial culture treatments, and measurements of different aspects of colony-level selection (trait variation, fitness, among others).

• *IGE/SGE experiment*: these consisted of controlled experiments done to assess the effects of IGE/SGEs on focal individuals phenotypic variation and/or fitness.

• *IGE/SGE breeding*: these studies consisted on breeding programs that incorporated the calculation and effects of IGE/SGEs.

• *Psychology experiment*: these were psychological experiments following and aimed to assess a cultural multilevel selection framework (Wilson et al., 2023).

• *Breeding through group selection*: typically, these studies have two contrasting breeding treatments: individual-based breeding (classical way to breed animals or crops) and group-based breeding, measuring the individual and group phenotypic effects and productivity of both treatments after several generations

• *Artificial selection*: in these studies, humans selected whole communities (like microbiomes) or species consortia or aggregates (like yeast aggregates) for specific desired traits (for example, like bigger colony size for yeasts), under specific environmental conditions. For example, studies implementing artificial selection for multicellularity in yeasts (Ratcliff et al., 2012; Bozdag et al., 2023) match this category.

These 16 categories were created by organizing all qualifying MLS empirical papers by similarity and/or main topic and/or main method assessed. The 67 sub-categories are mostly related to specific taxa or study systems, techniques, or sub-topic (**Table 1**; further information in **Supplementary Material; Zenodo:** https://doi.org/10.5281/zenodo.16633276).

Contextual analysis studies

Lastly, with the specific goal of comparing the strength and direction of natural selection as measured across different levels of biological organization, we conducted a detailed analysis of the 25 phenotypic selection studies that explicitly measured selection at multiple levels of biological organization (individual organisms and demes). Specifically, we extracted the available beta regression coefficients of each study, as these coefficients depict the direction and strength of selection on the trait in question at individual and group levels. The complete dataset of these coefficients is found in **Supplementary Table S2** (**Supplementary Material; Zenodo:** https://doi.org/10.5281/zenodo.16633276).

Results

The identification phase of the Scopus search yielded a total of 2,950 articles (after deleting duplicates) (**Fig. 1**). A total of 1,829 papers remained after exclusion of all articles not related to biology, cooperation, and social behavior in general (screening phase). From these, only 414 papers included empirical studies and thus persisted in the eligibility phase (**Fig. 1**). Finally, 166 articles indicating group selection or MLS as possible or plausible mechanism but not ensuring it as an explanation, were also excluded, resulting in a total of 248 papers providing empirical support for MLS found with Scopus. The additional search with Google Scholar, which was restricted to Web of Science-indexed articles, added 32 articles to this list, leading to a total of 280 scientific articles providing empirical support for MLS (**Fig. 1**).

These articles spanned 1976 – 2024, and 180 consisted of laboratory-controlled experiments, while the remaining 100 consisted of *in situ* (field) measurements and/or experiments (**Fig. 2**). Only years 2019, 2021, and 2023, yielded 20 or more MLS empirical papers, with a peak of 22 studies in 2019 (**Fig. 2**). Only 81 studies were published during the first 35 years of MLS empirical research (1976 – 2011), while the remaining 199 have been published since 2012, showing a marked increase in research in the last 12 years, both on MLS in situ and experimental studies (**Fig. 2**).

Regarding the taxa or study systems, systems like farm animals, eusocial insects, 'other insects' (this means non-eusocial insects such as beetles, spiders, water striders, among others), and humans, together compose approximately 65% and 55% of experimental and MLS *in situ* studies, respectively (**Fig. 3**). However, in MLS *in situ* studies, systems like plants, wild mammals, and wild birds also make up an important proportion of studies, while this is the case for bacteria and fungi in MLS experimental studies (**Fig. 3**). Many other study systems or taxa have been empirically investigated under a MLS framework: tunicates, polychaetes, viruses, crops, algae, fish, microbiomes, etc. (**Fig. 3**).

Regarding the levels of biological organization investigated, 90.4% of MLS empirical studies focused on individual organisms and groups of organisms. In particular, 71% of studies (198 papers) focused on demes, while 19.4% of studies focused on tighter organismal groups: 24 studies were conducted at the 'aggregate' level (aggregates of bacteria, amoebas, algae, and yeast) and 31 studies investigated colony-level selection (mostly in eusocial insects but also including spider and *Caenorhabditis elegans* colonies studies). A 9.6% of MLS empirical studies focused on organization levels above or below organisms/groups of organisms: four studies were conducted at the cell level (this include horizontal gene transfer or RNA viruses, for example); three studies were conducted at the genetic element level (specifically investigating selfish genetic elements or gene transfer agents using an MLS framework); 13 studies investigated community-level selection (mostly microbiomes, but also including beetles, ants, and arthropod communities); three studies with either algae or seagrass investigated clonal or module-level selection, i.e. selection acting at the clonal level; two studies with fungi investigated natural selection at the nuclei level, as some fungal taxa can contain thousands of nuclei on a single spore; and finally, two studies investigated natural selection at the species level.

Both *in situ* and experimental MLS empirical evidence comes from many different sources, types of study, and taxa or study systems, to the point that our 16 main categories were sub-categorized into 67 sub-categories (**Table 1; Supplementary Material; Supplementary Data; Zenodo:** https://doi.org/10.5281/zenodo.16633276). More than half (n=54) of MLS *in situ* studies used either IGE/SGE's measurements or contextual analysis, with categories such as cultural multilevel selection (n=10), group effects (n=9), and molecular sequencing (n=8) also having important numbers (**Table 1)**. Similarly, 84 of the 180 MLS experimental studies were laboratory experiments of different types, with the group selection treatments on wild animals sub-category in particular having 18 studies (**Table 1; Supplementary Material; Zenodo:**

https://doi.org/10.5281/zenodo.16633276). Other MLS experimental studies categories also had an important number of articles, including IGE/SGE experiment (n=23), artificial selection (n=22), and breeding through group selection (n=21). **Box 2** gives a brief summary of 16 representative studies of each one of the 16 main categories.

Finally, regarding the 25 studies that implemented contextual analysis in natural populations, it was not possible to extract the regression coefficient information from six of them (**Supplementary Table S2, Supplementary Material; Zenodo:** https://doi.org/10.5281/zenodo.16633276). Thus, **Fig. 4** shows the regression coefficients from 19 studies spanning 1995 – 2023, which were conducted in a plethora of study systems: from plants and water striders to chipmunks and humans. In **Fig. 4**, the effects of individual ('size') and group (average 'size' of the neighborhood individuals) traits on focal individuals' fitness is shown with the Beta (β) regression coefficients. In some studies (i.e. Stevens et al., 1995), group selection is stronger and goes in an opposite direction than individual selection, while in other studies (i.e., Donohue, 2004) the strength and direction of individual and group selection are similar, and in other studies (i.e., Bolstad et al., 2012), individual selection is significantly stronger than group selection (**Fig. 4**). In summary, there is a variety of selection outcomes across the 19 studies as revealed by contextual analysis, with some showing selection at different levels acting in concert while others show selection acting in opposition (**Fig. 4**).

Discussion

Here we show that contrary to common misconceptions, there is vast empirical evidence of multilevel selection (MLS) both *in situ* and with experimental studies, spanning five decades. Such evidence encompasses a broad spectrum of study systems and taxa, albeit systems like farm animals, eusocial and non-eusocial insects, and humans have been the main focus of MLS research. Similarly, and likely due to the organismal focus of most biologists, but also due to methodological feasibility, individual organisms and groups of organisms (demes, colonies, aggregates) have been the most investigated levels of selection in the MLS empirical research literature. With our analysis we can conclude that there is not a single or majority way to investigate MLS *in situ* or experimentally. Rather, multiple tools or ways of empirically investigating MLS have been used through the decades, which respond to the specificity of each study system or taxa, level of organization, and/or topic. Further, our bibliometric screening shows that from 1,829 articles that deal in some way with MLS or social evolution, 1,415 articles (77%) consisted of mathematical and conceptual models (**Fig. 1**), opinion pieces, debates, reviews, simulations, and so on. These are important in their own right but are excluded here because we are concerned with the realized utility of the MLS framework in empirical research. Group selection was initially rejected, not due to evidence, but due to its supposed theoretically implausibility (e.g., Williams, 1966; Maynard Smith, 1964). The large number of models demonstrating the theoretical plausibility of MLS therefore complements our review of the empirical literature.

The debate on the units of selection has gone on for too long. It is high time to move on and focus on the empirical evidence and data (Marín, 2015, 2016, 2024). Because there is a plurality of levels of selection investigated, from selfish genetic elements and nuclei to microbiomes, not all tools or experiments will work the same. For example, contextual analysis has been shown to work well under the Evolutionary Change School (Goodnight et al., 1992), but because of different model assumptions, does not work under the direct fitness (Hamiltonian) approach (Goodnight, 2013). Similarly, it would be quite challenging to apply contextual analysis in artificial selection experiments dealing with multicellularity evolution. Following our comprehensive MLS definition (**Box 1**), here we focused on compiling an extensive list of studies showing either differential reproduction of entire groups, or the differential reproduction of individuals being affected by their group composition or characteristics.

The plurality of study types and systems involves a variety of different methods to assess MLS *in situ.* For example, some *in situ* studies infer MLS by using molecular sequencing tools such as microsatellites, fingerprinting, and genome-wide association studies (**Table 1**), among other tools. More than half of MLS in situ studies implemented either IGE/SGE assessment or contextual analysis (**Fig. 4)**, finding quite significant effects of the neighborhood genes or traits or emergent traits on focal individuals' fitness (and individual trait variation). Such neighborhood/emergent effects are a core feature (**Box 1**) of MLS, with IGE/SGE's, contextual analysis, and group effects measurements, representing different ways in which they are calculated. It is not within the scope of this article to compare such mechanisms of assessment, as this has been done plenty in the literature (i.e., Bijma & Wade, 2008; Goodnight, 2013). In particular, Bijma and Wade (2008) have shown the relationships between kin selection, MLS, and IGE's. Rather, here we show that when group composition or characteristics or average/emergent traits are considered in the response to selection models' (in addition to individual traits), focal individuals' fitness are affected by such group composition or characteristics. This is supported by recent meta-analyses by Santostefano et al. (2025) and Burch et al. (2024), which respectively showed that IGE and epistasis are ubiquitous across the Tree of Life.

To understand whether natural selection occurs across different levels of biological organization (MLS), it is necessary to understand what it is meant by the term 'unit of selection'. Historically, MLS models can be characterized in terms of two schools of thought which differ in their conception of the units of natural selection: the unifying project and the disambiguating project (Suárez & Lloyd, 2023; Marín, 2024). In the unitary project (Lewontin, 1970), the expression “units of selection” has a unique meaning defined by features of the process of natural selection and all levels of selection share these features. Thus, this framework aims to find entities across the biological hierarchy that possess phenotypic variation, differential reproduction, and inheritance. In contrast, in the disambiguating project (Lloyd, 1983, 2024), the “units of selection” at any given level in the biological hierarchy can have one or more of three functional roles in the process of natural selection, which must be distinguished from each other. These roles are interactors, replicators/reproducers/reconstitutors, and manifestors of accumulated adaptations/type-1 agents (see Suárez & Lloyd, 2023).

An interactor is an entity that interacts *directly* with its environment through its traits with the result that the proliferation of interactors is differential. That is, interactors vary phenotypically from one another and, as a result, have differential reproduction or differential proliferation due to differences stemming from the interactor trait-environment interactions (Lloyd, 2024; Marín & Wade, 2025). Interactors are the entities that directly experience natural selection. The replicators or reproducers or reconstitutors are the entities that are differentially copied (replicator), differentially transmitted through material overlap (reproducer), or differentially recreated in the absence of copy or material overlap (reconstitutor) across generations (Suárez & Lloyd, 2023), as a result of the differential proliferation of interactors. For centuries, naturalists have been interested in documenting accumulated adaptations, such as the human eye or the beaks of Darwin’s finches. Biological entities that have such accumulated adaptations, have been deemed as ‘Manifestors of adaptation’ (Suárez & Lloyd, 2023). It is worth noting that many (if not most) adaptations are not accumulated: rather, most are ‘product of selection’ adaptations, in which their proportion changes through generations but not their biology. A classical example of a product of selection adaptation is the industrial melanism in the moth *Biston betularia* (L.) (Steward, 1977). The manifestors of accumulated adaptations resulting from differentially reproducing interactors need not be the interactors themselves. Similarly, although in some instances, interaction and replication can occur at the same level of biological organization -like with selfish genetic elements or during evolutionary transitions in individuality (Suárez & Lloyd, 2023)- most often they occur at different levels.

The comprehensive definition of MLS that we employ here (**Box 1**) falls into the disambiguating project of the units of selection literature. Thus, for a biological entity to be considered a unit of selection, two minimal things are required: phenotypic variation and differential reproduction. Furthermore, Suárez and Lloyd (2023; p. 17) have defined natural selection as a “process in which the differential proliferation of interactors causes the differential replication of replicators” (or the differential reproduction of reproducers or the differential reconstitution of reconstitutors). This clarification is necessary, as many of the historical (Williams, 1966) and current-day (Harms et al., 2023) critiques of MLS confound the roles of the different units of selection (Gould & Lloyd, 1999), requiring replication/reproduction/reconstitution (inheritance) of a biological entity to be considered as a unit of selection. This is not necessarily the case. For example, although typically genes constitute replicators, in specific cases such as selfish genetic elements, under such cases genes *might* also be considered as interactors (Gitschlag et al., 2020).

The comprehensive definition of MLS (**Box 1**) employed here captures instances in which entire groups constitute the inheritance unit (replicator/reproducer/reconstitutor) and instances in which entire groups constitute the interactor unit but the inheritance unit is at a lower level of biological organization (most typically, the individual organism or its genetic material). The latter cases are typically detectable with techniques such as IGE’s measurements, social network analysis, the Price equation, and contextual analysis, among others (Marín & Wade, 2025), as mentioned above. In summary, MLS occurs when natural selection operates simultaneously among two or more different levels of a nested biological hierarchy, which either causes differential reproduction of entire groups (i.e., the group is also the replicator/reproducer/reconstitutor) or when the differential reproduction of individuals is influenced by their group composition or its characteristics (i.e., lower-level entities are the replicator/reproducer/reconstitutor) (**Box 1**).

In the debate about units of selection, there have been strongly gene-centric (Marín & Wade, 2025) and adaptationist (Marín, 2024) biases, which are tightly related. The conceptual foundations of the gene-centric view are models of non-structured populations so large that all combinations of genes, individuals, and environments are entirely random, a situation difficult to reconcile with most of the biological world (Marín & Wade, 2025). A gene’s average effect on phenotype and fitness, even in an unstructured population, depends upon the trait values of parental genotypes (or genotype combinations) and allele frequencies, and so cannot be measured directly, unlike the breeding value of an individual (see Falconer, 1981). Moreover, in a metapopulation, the average effect of a gene is defined locally and will vary among localities depending on its interactions with other genes (epistasis), other individuals (e.g., social interactions), the microbiome (epistasis between genomes), and the local environment (genotype by environment interactions), and this constellation of contexts may itself change between generations, populations, and environments (Marín & Wade, 2025). Where the ‘Adaptationist school of evolutionary thought’ (Goodnight & Stevens, 1997), does not consider epistatic and other interactions as important or significant, the

‘Evolutionary change school’ (Marín, 2024) emphasizes interactions of all kinds, especially those involving epistasis between genes and between genes and the social environment as they affect individual fitness. This is important because epistatic interactions, which are a main feature of MLS, have been shown to ubiquitous across the Tree of Life in a recent meta-analysis of 1606 trait datasets (Burch et al., 2024).

The pioneering study by Wade (1976) (**Box 2**) was the starting point of laboratory studies on which group selection was imposed as a treatment. Several dozen similar studies (imposed group selection in laboratory populations) were conducted through the decades (**Table 1, Fig. 2**), always finding rapid responses to the group selection treatments after a few generations. Further, such imposed group selection studies found that selection sometimes acts in concert and sometimes in opposition at the individual and group levels, also with varying strength. Interestingly, the same pattern is found when analyzing contextual analysis studies (**Fig. 4**): natural selection sometimes acts at the same and sometimes at different directions and strengths across levels of biological organization. As such, no generalization can be made about MLS and it should be investigated on a case by case manner (Wilson & Wilson, 2007; Eldakar & Wilson, 2011). However, ecological constraints can help predict responses to selection. For example, when in 2017 the category 4 Hurricane Maria almost totally destroyed a Puerto Rican island inhabited by rhesus macaques, shade became a very scarce resource. As a response, there was a marked increase in tolerance and decrease in aggression among macaques (Testard et al., 2024), with the most tolerant animals having the highest survival. Similarly, in plant-mycorrhizal associations it has long been known that under scarcity of nutrients (particularly nitrogen and phosphorous), this symbiotic association becomes more mutualistic while under ‘luxury’ conditions (excess of nutrients), the usually benign mycorrhizal fungal microbiomes can behave as nutritional parasites (Johnson et al., 1997; Johnson & Marín, 2024).

Several other influential MLS experimental studies include Craig and Muir (1996), Swenson et al. (2000), Ratcliff et al. (2012), and Bozdag et al. (2023). Craig and Muir (1996) and several dozen more studies (a total of 32 studies; see **Table 1**: 3. IGE/SGE breeding and 4. Breeding through group selection) have shown that MLS is a very useful framework for breeding programs of farm animals and crops. Furthermore, when farm animals or crops are bred through group selection treatments (i.e., selecting group traits) or when IGE/SGE’s are considered in breeding programs, the outcome is always the desired for the farmer: higher yields or more production. Even MLS skeptics recognize the value of MLS-focused breeding programs in wheat cultivars (Zhu et al., 2019a, b, 2022). Empirical evidence showing the success of wheat breeding for higher yields over the past 100 years in northwestern China has been argued to result in part from “unconscious group selection on root traits" (Zhu et al., 2019a), which results in smaller, less branched, and deeper roots.

Swenson et al. (2000) pioneered the framework of artificial ecosystem selection as a way of selecting communities of soil microorganisms based on plant performance. This implies exposing multiple generations of plants to particular selection pressures, selecting the microbiomes that increase plant fitness (or selected traits) to the next generation, while the genetic basis of the host remains the same. This approach has been successfully used to engineer belowground communities that increase plant tolerance to drought (Lau & Lennon, 2012; Jochum et al., 2019) and salinity (Mueller et al., 2021), or that increase leaf greenness (Jacquiod et al., 2022), among others (reviewed in Sanchez et al., 2021, 2023 and Yu et al., 2023). On their part, Ratcliff et al. (2012) and Bozdag et al. (2023), implementing artificial selection regimes in yeast aggregates, have shown some of the most visually stunning examples of experimental MLS: they shown *de novo* evolution of macroscopic multicellularity just after one year and 600 rounds of selection (Bozdag et al., 2023). In particular, in an anaerobic treatment, yeast evolved to be macroscopic, becoming 2×10^4 times larger than at the beginning, while maintaining a clonal multicellular life cycle (Bozdag et al., 2023).

A MLS framework has long been used to investigate human culture (Soltis et al., 1995), originating a whole sub-discipline, deemed 'cultural multilevel selection' (Wilson et al., 2020, 2023). In our review, a total of 30 MLS empirical studies were centered around humans: 19 consisted of psychological experiments, 10 assessed or inferred cultural MLS *in situ*, and one implemented contextual analysis over 55 years of polygyny and polyandry data, based on the Utah Population Databas (Moorad, 2013). MLS seems to explain the most important cultural macroevolutionary patterns and historical trends, including competition and warfare but also exchange and selective imitation (Turchin & Gavrilets, 2021; **Box 2**). The utility of MLS has been recognized in anthropology: a survey to 175 evolutionary anthropologists (faculty members of graduate programs) finds that 78.7% of them regard cultural MLS as "important", while 64.9% disagree with the statement "Group selection has no useful role to play in social science" (Yaworsky et al., 2015). Whether a similar acceptance rate of MLS by evolutionary biologists not working with humans is yet to be analyzed/surveyed.

Our findings showing a marked increase in MLS research in the last 12 years (**Fig. 2**), with 199 MLS studies since 2012, indicates both that MLS is becoming more accepted as a conceptual framework and that many studies are using adequate sample sizes to ask questions across levels of biological organization. With the marked increase since 2012 and expanding acceptance of MLS as an conceptual evolutionary framework, many more groundbreaking studies are to come in the next few decades.

There are some caveats to our findings that the evidence for MLS is vast. First, we expect a publication bias towards studies finding positive outcomes, by which we mean that some studies where no selection at a higher level was found, were likely not captured. Despite this, our database does include studies in which higher-level selection or group properties were not important in explaining focal individual trait variation and fitness (Philson & Blumstein, 2023a, b). Further, in several of the contextual analysis studies (Tsuji, 1995; Donohue, 2003; Weinig et al., 2007; Boege, 2010; Eldakar et al., 2010; Fornica et al., 2011; Bolstad et al., 2012; Laiolo & Obeso, 2012; Fisher et al., 2017, 2021) (**Fig. 4**), the magnitude of selection was stronger at the individual than at the group level. Similarly, direct genetic effects are also usually stronger than indirect genetic effects, as shown by the meta-analysis of Santostefano et al. (2025) and through our database (but see Santostefano et al., 2021). However, because MLS should be evaluated in a case-by-case scenario (Wilson & Wilson, 2007), this is not problematic for our framework: depending on the environmental context, case, and traits, it is expected that there will be cases in which there are no group effects or they are not as important as individual-level effects. Secondly, in order to have a distinct cutoff, we excluded MLS empirical evidence produced after 2024, thus missing new studies such as Philson et al. (2025), showing the first evidence for MLS on individual- and group-level vertebrate social behavior in the wild.

In general, we were quite strict in our search. For example, a study classically cited by some as the first MLS empirical study (Lewontin, 1962) was excluded, because, although it is based on real lab mice population data, the conclusions (about interdemic selection) are based on Monte Carlo simulations. Similarly, studies arguing that MLS is a ‘likely’ (Dyer et al., 2005) or ‘possible’ (Aviles, 1986) explanation were also excluded. Thus our total of 280 articles obtained is an underestimate of the evidence and conceptual use, because many more studies that clearly show results consistent with the MLS framework (i.e., Pope, 1992; Heinsohn & Packer, 1995; Ingvarsson, 2000; Papkou et al., 2023; Barnett et al., 2025), have historically avoiding using the term (Eldakar & Wilson, 2011). For example results based on Wright’s fitness landscapes (Papkou et al., 2023) or on evolvability (Barnett et al., 2025), explicitly require a MLS perspective to understand them. Although a MLS framework may not be explicitly mentioned by name, and in some cases may be avoided due to historical misconceptions (Eldakar & Wilson, 2011), it is implicit in experimental design and rationale.

In summary, a thorough search of the literature shows that contrary to common misconceptions which plagued the field since the 1960’s, there is vast empirical evidence of multilevel selection (MLS) both in situ and via experimental studies. We found 280 papers providing empirical support for MLS; 100 *in situ* and 180 laboratory experiments. The studies span many taxa and research methodologies, meaning MLS is not situational or an exception: MLS is a powerful evolutionary force in nature. Disregarding MLS will continue to hold the field of evolutionary biology back and prevent us from more fully understanding life on earth.

Data availability statement

The database used for this Review is available at Zenodo: https://doi.org/10.5281/zenodo.16633276

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Conflict of interest

The authors declare no conflict of interest.

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Box 1: Glossary of terms (underlined in the text)

- **Artificial selection**: Human goal-driven selective breeding. Humans breed whole communities (like microbiomes) or species consortia or aggregates (like yeast aggregates) for specific desired traits (like bigger colony size, for yeasts), under imposed environmental conditions.
- **Breeding through group selection**: Artificial selection where humans control the context for reproduction in such a way as to influence how groups of organisms function (e.g. reduced competition). Typically, these studies have two contrasting breeding treatments: individual-based breeding (classical way to breed animals or crops) and group-based breeding. 'Group-based breeding' means that emergent or contextual or group-level traits are the basis for the breeding program.
- **Contextual analysis**: Contextual analysis follows the methods for analyzing phenotypic selection originally developed by Lande and Arnold (1983) and Arnold and Wade (1984), where a multiple regression of relative fitness on phenotype is performed (Goodnight et al., 1992). Contextual analysis extends such methods by including "contextual" or "emergent" traits, that is, traits measured on the group or neighborhood, in the multiple regression. In this way, relative fitness is a function of individual and group or emergent traits. This phenotypic selection tool allows to disentangle the strength and direction of selection operating at the individual and group levels. Goodnight et al. (1992) has shown that contextual analysis is an useful tool, compatible with models that explicitly include differential extinction of entire groups (Levins, 1970), Wright's definition of interdemic selection – which does not require group extinction (Wright, 1945), and trait-group models Wilson (1975).
- **Cultural multilevel selection**: Multilevel selection in which the inheritance system is cultural transmission, not genetic material. These studies investigated MLS in cultural traits, thus, for example these studies showed traits that confer a group-level advantage can spread via cultural MLS.
- **Indirect/social genetic effects (IGE/SGEs)**: An IGE has been defined as the "effect of a gene in the genome of one individual on the phenotype of another individual" (Wade, 2025). IGE's sometimes are also deemed as "social genetic effects" (SGE's). Recent meta-analyses on this subject were recently published by Santostefano et al. (2025) and Burch et al. (2024). Bijma and Wade (2008) have shown that when IGE's are included when calculating the response to selection, MLS without relatedness can explain the evolution of social traits.
- **Interdemic selection**: Variously defined, depending upon whether demes are relatively unbounded, if interbreeding subsections of populations or more organized subsections, equivalent to groups. In either case, the demes are usually assumed to exist across at least a generation and, for selection, to differ in productivity.
- **Multilevel selection**: Multilevel Selection (MLS) has been defined as a situation in which natural selection occurs among entities at two or more different levels in a nested biological hierarchy (Damuth & Heisler, 1988). Specifically, MLS occurs when there is differential reproduction of entire groups (as well as of individual entities within them), or when the differential reproduction of individuals is based on their group composition or characteristics.
- **Trait groups**: Trait groups (Wilson, 1975) are fitness-affecting associations between two or more individuals, regardless of the duration of the association or whether actual reproduction takes place. Selection is then acting on both individuals within groups and the groups or demes themselves.

Box 2: Representative studies of MLS *in situ* and experimental studies

MLS in situ

1. Cultural multilevel selection, Turchin and Gavrilets (2021). *Evol Psychol*: using a database of past societies history (*Seshat: Global History Databank*), the authors found that the tempo (rates of change) of cultural macroevolution is characterized by periods of apparent stasis interspersed by rapid change. They found that the most important macroevolutionary patterns include competition and warfare but also cultural exchange and selective imitation, fully in accordance with cultural multilevel selection theory.

2. *Molecular sequencing,* Yu et al. (2020). *Nat Ecol Evol*: this study implemented single nucleotide polymorphisms (SNP's) analysis to show that branching events in the seagrass *Zostera marina* clones or genets, lead to population bottlenecks of tissue that result in the evolution of genetically differentiated ramets in a process of somatic genetic drift. The authors found that thousands of SNP's segregated among ramets. This study provides "evidence for multiple levels of selection during the evolution of seagrass genets".

3. *Indirect Genetic Effects/Social Genetic Effects (IGE/SGE),* Santostefano et al. (2021). *Evolution*: the authors assessed how IGEs contributed to genetic variation of behavioral, morphological, and life-history traits in a wild Eastern chipmunk population, comparing the contribution of direct and indirect genetic effects to trait evolvability. They found significant IGE's for trappability and relative fecundity, but little direct genetic effects in all traits measured.

4. Group heritability, Walsh et al. (2020). *Am Nat*: the heritability, genetic correlations, and fitness consequences of three collective behaviors (foraging, aggression, and exploration) were estimated in the ant *Monomorium pharaonis*, as well as of body size, sex ratio, and caste ratio. The heritability estimates for the three collective behaviors were moderate (0.17 – 0.32), but lower than for caste ratio, sex ratio, and body size. Variation in collective behaviors among the different colonies was phenotypically correlated, indicating that selection shapes multiple colony collective behaviors at the same time.

5. Group effects, Bilde et al. (2007). *J Evol Biol*: the effects of group size on fitness components were investigated in the social spider *Stegodyphus dumicola* in two populations in Namibia. In both populations, an increased colony size resulted in improved survival of colonies and late-instar juveniles. Mean individual fitness was maximized in intermediate- to large-sized colonies. Thus, group living in these social spiders entails a trade off against survival benefits at the colony level.

6. Dataset analyses, Smith and Inglis (2021). *Proc R Soc B*: the authors surveyed 20 years of published scientific literature for mix experiments with different genotypes of the same microbial species, focusing on studies of social evolution. A total of 39 experiments matched the inclusion criteria, as these studies measured the asexual survival and reproduction of strains as a function of their initial frequency, holding constant the total number of individuals. The authors found that "strain and multilevel fitness outcomes were both effective for quantitatively comparing social selection in different datasets".

7. Contextual analysis, Stevens et al. (1995). *Am Nat*: this constitutes the first study to implement contextual analysis (Heisler & Damuth, 1987) in natural populations. This study partitioned selection into group and individual level components in natural populations of *Impatiens capensis*, measuring the relationships between three fitness components and several group and individual level traits. Two of the fitness components (survival rate and cleistogamous seed production) were affected by individual and group selection, while chasmogamous seed production (the third fitness component) was only affected by individual selection.

8. *Colony selection*, Robinson et al. (2023). *BMC Biol*: the ant *Rhytidoponera metallica* forms queen-less colonies, with such a low intra-colony relatedness that they are proposed as a transient, unstable form of eusociality. Despite this, these ants are among the most widespread in Australia, showing that relatedness is not necessary for such success. The authors show that these ants exhibits remarkable intra-colony variation regarding their polypeptidic venom composition (revealed by transcriptomic and mass spectrometry), with workers sharing only a relatively small proportion of toxins in their venoms. Such variation is not due to the presence of chemical castes, but is rather explained by toxin allelic diversity. The authors conclude that such high toxin diversity is explained through MLS, selecting for colonies that can exploit more resources and defend against a wider range of predators.

9. *Phylogenetics*, Herron and Michod (2008). *Evolution*: this study investigated the transition from unicellular to multicellular life in Volvocine algae. Phylogenetic reconstructions of ancestral character states were derived from the diverse array of extant species in the volvocine lineage ranging from unicellular to colonial forms that themselves vary in size, structure, and degree of cellular specialization. Herron and Michod (2008) describe an evolutionary history with multiple independent origins and reversals of traits that underlie cellular cooperation (i.e. transition of fitness from individual cells to the group level) as well as conflict-mediation mechanisms to curtail the exploitation of cooperation.

Box 2 (continuation)

10. *Field experiment*, McCauley (1994). *Am Nat*: this study assessed the relationship between aggregation behavior (mean crowding on the host plant) and mortality owing to a parasitoid fly in groups of the beetle *Leptinotarsa juncta*. Significant group-to-group variation in the propensity of beetles to aggregate was found in the laboratory, and also under field conditions. Further, three field studies were implemented to measure the relationship between aggregate and mortality due to the parasitoid. In an observational study (of naturally occurring populations), a significant positive relationship between group-specific survival and the aggregation degree was shown through a multiple regression (controlling for group size). In the other two field studies, group size and dispersion pattern were manipulated, also finding a significant positive regression of group-specific survival on degree of aggregation.

MLS Experimental

1. *Lab experiment*, Wade (1976). *Proc Natl Acad Sci USA*: first empirical study of MLS in our bibliometric search. Wade (1976) imposed group selection for both increased and decreased adult population size in laboratory populations of the beetle *Tribolium castaneum*, at 37 -day intervals. Individual selection control treatments (i.e. no group selection imposed) were included. Response to the group selection treatments occurred fast, at three or four generations, and in general was large in magnitude (some times 200% larger magnitude than the control).

2. *IGE/SGE experiment*, Baud et al. (2021). *Genome Biol*: in this study, the authors investigated IGE's in 1812 genetically heterogeneous laboratory mice (same sex, adults, unrelated, and housed in the same cage), by gathering a dataset of 170 behavioral, physiological, and morphological traits phenotypes measured in 1812 genetically heterogeneous laboratory mice to study IGE arising between same-sex, adult, unrelated mice housed in the same cage. Under such conditions, GWAS were applied, identifying IGE loci for 17 traits, and no overlap between IGE loci and direct genetic effects loci for the same trait.

3. *IGE/SGE breeding*, Ellen et al. (2008). *Poult Sci*: mortality due to cannibalism in laying hens depends on social interactions among individuals. This article presents estimations of IGE's and direct genetic effects on survival days in three purebred laying lines. To do so, they analyzed 16,780 hens with intact beaks. When only direct genetic effects were included, the heritailities ranged from 2% to 10%. When both direct genetic effects and IGE's were considered, the total heritable variance ranged from 9% to 19%. Thus, heritable variation in survival days is substantially larger than suggested by conventional direct effects models.

4. *Psychology experiment*, Francois et al. (2018). *Sci Adv*: this study provides evidence both from survey data and laboratory treatments of experimental subjects, consistent with a st of core concepts and theories based on cultural MLS. Specifically, the authors find that "increases in competition increase trust levels of individuals who (i) work in firms facing more competition, (ii) live in states where competition increases, (iii) move to more competitive industries, and (iv) are placed into groups facing higher competition in a laboratory experiment". They conclude that their findings provide support for cultural MLS as a contributor to human prosociality.

5. Breeding through group selection, Craig and Muir (1996), *Poult Sci*: an important behavioral problem with egg laying hens is their proclivity to aggressively peck their cage-mates. This can be minimized through the practice of beak-trimming; however, this can cause lasting pain for the animals involved, thus essentially improving one scenario of animal well-being at the cost of another. Craig and Muir (1996) investigated whether beneficial behaviors could be selected for at the group-level, thereby eliminating the need for beak-trimming. Three genetic stocks of hens were compared for mortality, injuries, and body condition: one of the lines involved the seventh-generation of group-selected hens (recurrent selection of the most productive cages), an unselected stock of hens, and a highly productive, typically beak-trimmed commercial stock. Overall, the group-selected lineage showed behavioral improvements over the unselected and commercial lines resulting in reduced cannibalism, better feathering, and improved welfare. Furthermore, when comparing the previous six generations of the group-selected line of collectively house hens to those housed individually (Muir, 1996), by the sixth-generation the collectively housed hens approximated the mortality of their solitary counterparts (8.8% to 9.1%, respectively). This was the result of a dramatic decrease in mortality from 68% in the second generation down to 8.8% in the sixth-generation of group-selected hens. In addition, the group-selected lineage also experienced substantial improvements in survival (from 169 to 348 days) and egg production per hen (from 91 to 237 eggs) over that same time frame.

6. *Artificial selection*, Bozdag et al. (2023). *Nature*: this multicellularity long-term evolution experiment was carried out with snowflake yeast (Saccharomyces cerevisiae), by selecting for larger group size under three metabolic treatments: anaerobic, obligately aerobic, and mixotrophic yeast. After 600 rounds of selection, yeast in the anaerobic treatment group evolved to be macroscopic, becoming around 2 × 104 times larger (about 1 mm, visible to the naked eye) and about 104-fold more biophysically tough, while retaining a clonal multicellular life cycle. Yeast in the aerobic treatment remained microscopic (only sixfold larger). This was explained through biophysical adaptation of increasingly elongate cells, which after some time facilitated branch entanglements that enabled groups of cells to stay together.

Table 1. Categories (16) and sub-categories (67) of topics/methods/assessments of multilevel selection (MLS) in situ and in Experimental studies. The number of papers per category/sub-category are presented in parenthesis. Total number of papers: 280.

MLS *in situ*
1. Cultural multilevel selection (No. of papers = 10; 1995 – 2023).
2. Molecular sequencing (No. of papers = 8; 2009 – 2024): Microsatellites (1); Gene Transfer Agents (2); Fingerprinting (1); Colonies' microsatellites (1); Colonies' genome-wide association studies (1); Clones' single nucleotide polymorphisms (1); Alleles for cooperation (1).
3. Indirect Genetic Effects/Social Genetic Effects (IGE/SGE) (No. papers = 29; 2008 – 2023): Wild animals (7); Farm animals (22).
4. Group heritability (No. papers = 2; 2020 – 2021): Wild animals (1); Farm animals (1).
5. Group effects (No. papers = 9; 2007 – 2024): Social networks – Wild animals (4); Selection – Wild animals (4); Group effects – Wild animals (1).
6. Dataset analyses (No. papers = 6; 1996 – 2022): Populations (2); Microbiomes (1); Hybrid zone (2); Communities (1).
7. Contextual analysis (No. papers = 25; 1995 – 2023): Unaltered – Humans (1); Unaltered (11); Manipulated (13).
8. Colony selection (No. papers = 3; 2023): Trait variation (1); Selection (1); Personality (1).
9. Phylogenetics (No. papers = 4; 1987 – 2015): Price equation (1); Heritability (1); Phylogenetics (2).
10. Field experiment (No. papers = 4; 1994 – 2019): Group effects – Wild plants (1); Group effects – Wild animals (2); Group effects – Crops (1).
Experimental MLS
1. Lab experiment (No. papers = 84; 1976 – 2024): Population heritability – Wild animals (1); Molecular sequencing – Virus' RNA (1); Molecular sequencing – Selfish genetic elements (1); Molecular sequencing – Colonies' microsatellites (1); Molecular sequencing – Alleles (1); Microbiome assessment – Wild animals (1); Group selection treatments – Wild plants (2); Group selection treatments – Wild animals (18); Group selection treatments – Virus (1); Group personality – Wild animals (1); Group fitness – Wild animals (1); Group effects (Social networks) – Wild animals (6); Group effects – Wild animals (14); Group effects – Farm animals (3); Group effects – Crops (2); Culture experiment – Fungi (1); Culture experiment – Bacteria (5); Culture experiment – Algae (1); Community selection treatments – Wild animals (2); Community heritability – Wild animals (1); Colony trait variation – Wild animals (8); Colony selection – Wild animals (8); Colony fitness – Wild animals (2); Clonal lineages – Fungi (1); Clonal lineages – Algae (1).
2. IGE/SGE experiment (No. papers = 23; 1987 – 2023): Wild animals (14); Plants (1); Microorganisms (1); Lab mice (1); Farm animals (6).
3. IGE/SGE breeding (No. papers = 11; 2008 – 2019): Wild animals (1); Farm animals (9); Crops (1).
4. Psychology experiment (No. papers = 19; 2011 – 2024).
5. Breeding through group selection (No. papers = 21; 1996 – 2023).
6. Artificial selection (No. papers = 22; 2000 – 2023): Single species (4); Multicellularity (12); Microbiome (5); Consortia (1).

Further explanations of each category and sub-categories are given in **Supplementary Material** (**Zenodo:** https://doi.org/10.5281/zenodo.16633276).

Figure legends

Figure 1. BIBLIO flow diagram for bibliometric review of empirical studies of multilevel selection. *Excluded areas in the SCOPUS search: Dentistry; Nursing; Energy; Chemical Engineering; Health Professions; Pharmacology, Toxicology and Pharmaceutics; Business, Management and Accounting; Materials Science; Physics and Astronomy; Engineering; Computer Science; Arts and Humanities; Mathematics; Medicine.

Figure 2. Number of Web of Science/Scopus-indexed articles (n=280) published between 1976 and 2024 providing empirical support for multilevel selection *in situ* (n=100; blue) and through Experimental studies (n=180; orange).

Figure 3. Proportion of study systems for multilevel selection in situ (MLS in situ) and Experimental studies.

Figure 4. Summary of 19 (out of 25) contextual analysis of phenotypic selection done between 1995 and 2023. Beta regression coefficients show the effects of organismal ‘size’ (or traits which are a proxy of size, like height) at the individual (black dotes) and group (red dotes) levels, on individual fitness (or fitness proxies).

Figure 1.

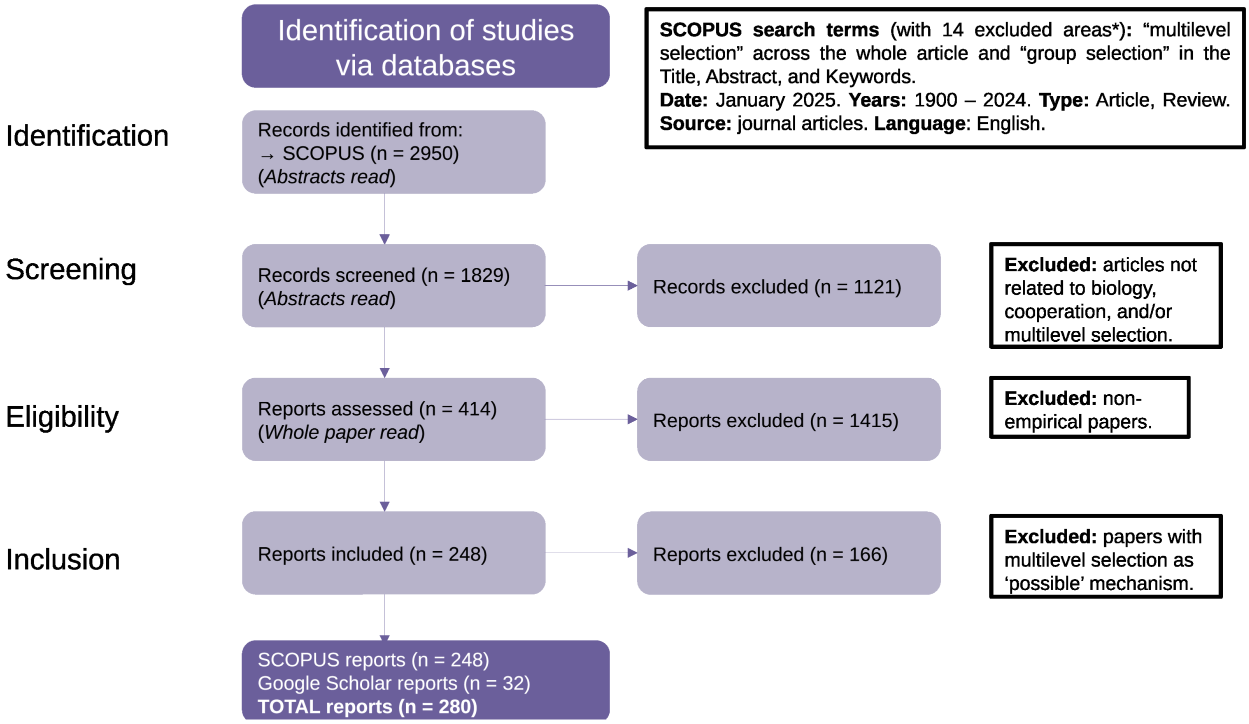
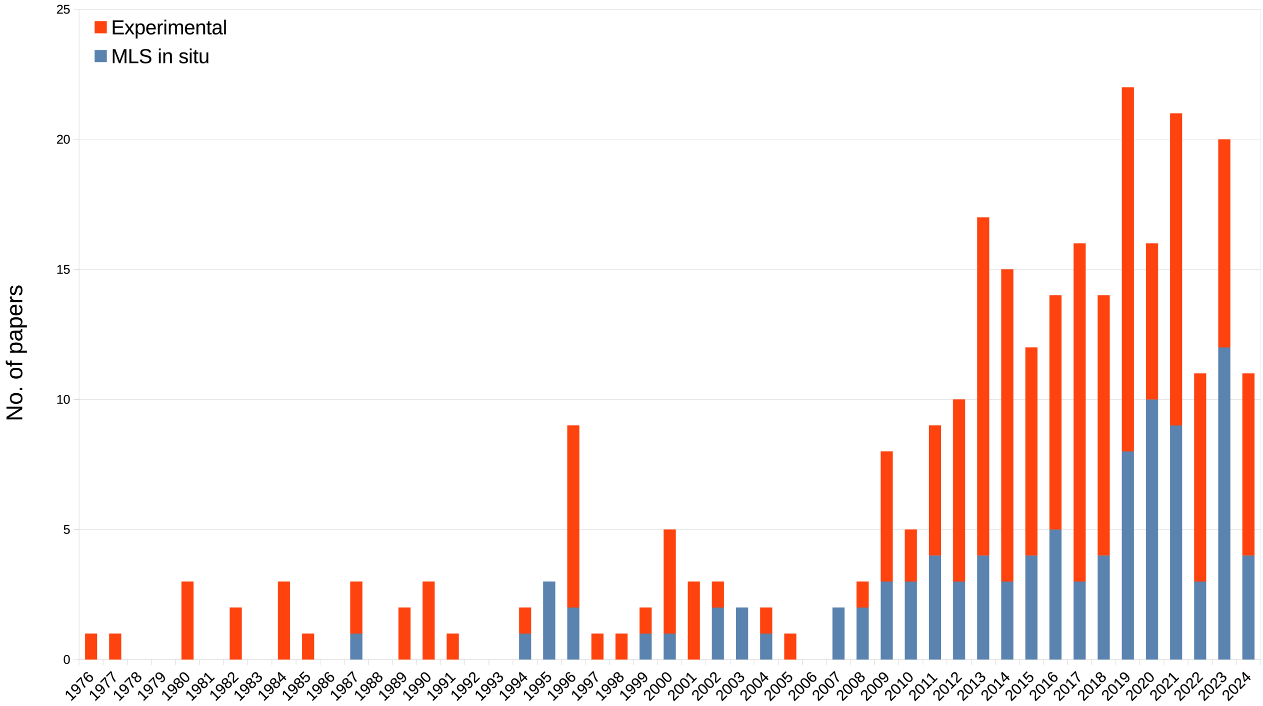

Identification of studies via databases
SCOPUS search terms (with 14 excluded areas*): "multilevel selection" across the whole article and "group selection" in the Title, Abstract, and Keywords. Date: January 2025. Years: 1900 – 2024. Type: Article, Review. Source: journal articles. Language: English.
Identification
Records identified from: → SCOPUS (n = 2950) (Abstracts read)
Screening
Records screened (n = 1829) (Abstracts read)
Records excluded (n = 1121)
Excluded: articles not related to biology, cooperation, and/or multilevel selection.
Eligibility
Reports assessed (n = 414) (Whole paper read)
Reports excluded (n = 1415)
Excluded: non-empirical papers.
Inclusion
Reports included (n = 248)
Reports excluded (n = 166)
Excluded: papers with multilevel selection as 'possible' mechanism.
SCOPUS reports (n = 248)
Google Scholar reports (n = 32)
TOTAL reports (n = 280)

Figure 2.


Experimental
MLS in situ
No. of papers
0
5
10
15
20
25
1976 1977 1978 1979 1980 1981 1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024

792 **Figure 3.**

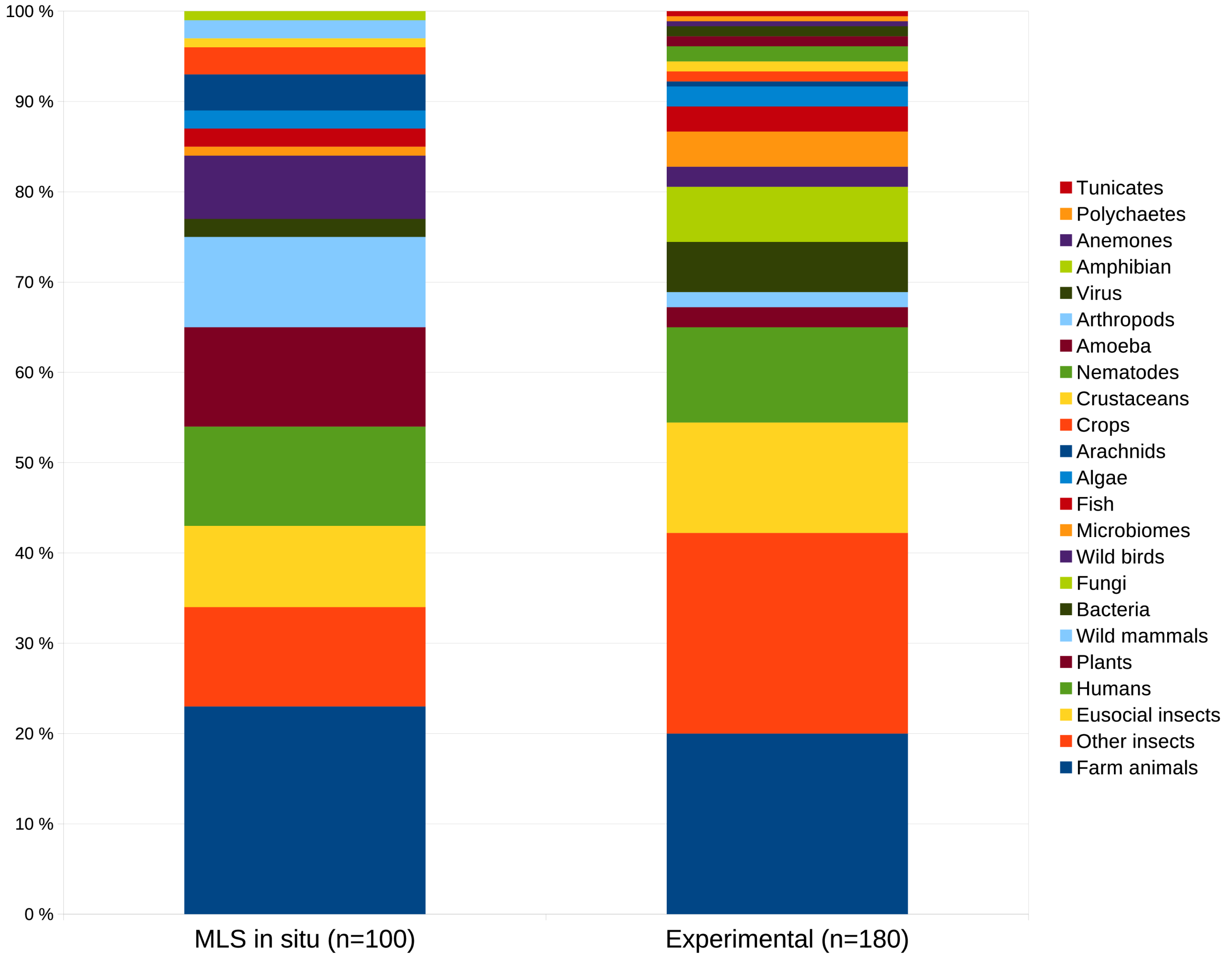
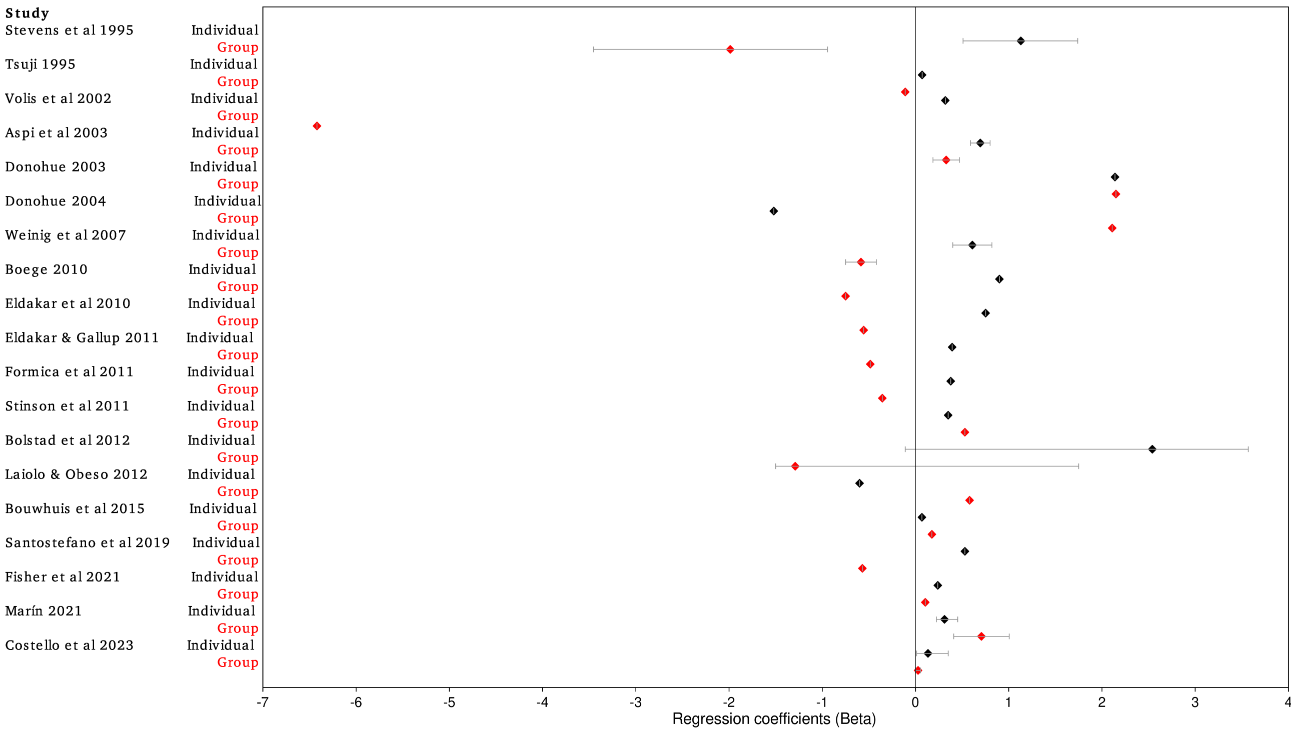

100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
MLS in situ (n=100)
Experimental (n=180)
Tunicates
Polychaetes
Anemones
Amphibian
Virus
Arthropods
Amoeba
Nematodes
Crustaceans
Crops
Arachnids
Algae
Fish
Microbiomes
Wild birds
Fungi
Bacteria
Wild mammals
Plants
Humans
Eusocial insects
Other insects
Farm animals

Figure 4.


Study
Stevens et al 1995
Tsuji 1995
Volis et al 2002
Aspi et al 2003
Donohue 2003
Donohue 2004
Weinig et al 2007
Boege 2010
Eldakar et al 2010
Eldakar & Gallup 2011
Formica et al 2011
Stinson et al 2011
Bolstad et al 2012
Laiolo & Obeso 2012
Bouwhuis et al 2015
Santostefano et al 2019
Fisher et al 2021
Marín 2021
Costello et al 2023
Individual
Group
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4
Regression coefficients (Beta)