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Insects in Scientific Research: A Philosophical Examination through the Lens of the 3Rs

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Abstract

This essay examines the ethical implications of using insects in scientific research through the framework of the 3Rs—Replacement, Reduction, and Refinement. Historically, ethical considerations in research have focused on vertebrates, but increasing evidence suggests that insects may possess more complex cognitive and sensory capacities than previously thought, raising moral questions about their treatment.

The principle of Replacement is discussed in relation to alternative methods such as in silico models, in vitro systems, and biomimetics, which offer promising ways to reduce the reliance on live insects. However, these alternatives are not yet advanced enough to fully replicate the complexity of biological processes in insects. Reduction, which aims to minimize the number of animals used in research, requires more precise statistical techniques and better experimental design to balance ethical concerns with scientific rigor. Finally, Refinement emphasizes minimizing suffering and improving welfare, including the use of anesthesia and appropriate euthanasia techniques, although research into insect welfare and euthanasia methods remains limited.

Through an analysis of utilitarianism, deontological ethics, and virtue ethics, this essay argues that ethical considerations must extend to insects. Despite their differences from vertebrates, the 3Rs should guide insect research to reduce harm and promote responsible scientific inquiry.

Keywords: Insect research ethics, 3Rs (Replacement, Reduction, Refinement), Animal welfare, Sentience and suffering.

Τα έντομα στην επιστημονική έρευνα: μια φιλοσοφική προσέγγιση υπό το πρίσμα των 3Rs

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Περίληψη

Η παρούσα εργασία εξετάζει τις ηθικές προεκτάσεις της χρήσεως εντόμων στην επιστημονική έρευνα υπό το πρίσμα των 3Rs—Αντικατάσταση (Replacement), Μείωση (Reduction) και Βελτίωση (Refinement). Ενώ οι ηθικές συζητήσεις στην επιστημονική έρευνα έχουν επικεντρωθεί κυρίως στα σπονδυλωτά, όλο και περισσότερα δεδομένα δείχνουν ότι τα έντομα διαθέτουν πιο σύνθετες γνωστικές και αισθητηριακές ικανότητες από ό,τι θεωρούνταν μέχρι πρότινος. Επομένως, προκύπτουν σοβαρά ηθικά ζητήματα για την ορθή διαχείρισή τους στην έρευνα.
Η Αντικατάσταση (Replacement) εξετάζεται μέσα από εναλλακτικές μεθόδους, όπως μοντέλα in silico, συστήματα in vitro και βιομιμητική, που προσφέρουν λύσεις για τον περιορισμό της εξαρτήσεως από ζώντα έντομα. Εν τούτοις, οι μέθοδοι αυτοί δεν έχουν ακόμη εξελιχθεί προς την πλήρη αντικατάσταση των βιολογικών διεργασιών των εντόμων. Η Μείωση (Reduction) στοχεύει στον περιορισμό του αριθμού των εντόμων που χρησιμοποιούνται στην έρευνα, αξιοποιόντας βελτιωμένες στατιστικές τεχνικές και πειραματικό σχεδιασμό. Τέλος, η Βελτίωση (Refinement) επικεντρώνεται στην ελαχιστοποίηση του πόνου και στην ευζωία των εντόμων, όπως με τη χρήση αναισθησίας και κατάλληλων μεθόδων ευθανασίας, αν και η έρευνα στον τομέα αυτόν παραμένει περιορισμένη.
Μέσα από μια ανάλυση του ωφελιμισμού (utilitarianism), της δεοντολογικής ηθικής (deontological ethics) και της αρεταϊκής ηθικής (virtue ethics), η παρούσα μελέτη υποστηρίζει οτι οι ηθικές εκτιμήσεις πρέπει να επεκταθούν και στα έντομα. Παρ' όλο που διαφέρουν από τα σπονδυλωτά, η εφαρμογή των 3Rs μπορεί να περιορίσει την επιβάρυνση, και να συμβάλει σε μια πιο υπεύθυνη επιστημνική έρευνα.

Keywords: Ηθικὴ της έρευνας με έντομα, 3Rs (Αντικατάσταση, Μείωση, Βελτίωση), ευζωία ζώων, αισθαντικότητα και πόνος.

1. Introduction

In scientific research, the ethical treatment of animals has been governed by the principles of Replacement, Reduction, and Refinement (the 3Rs). These principles aim to minimize the use of animals and to mitigate harm where their use is necessary. However, most discussions about the ethical treatment of animals in research focus on vertebrates, often overlooking invertebrates, particularly insects.

As insect research expands, it is crucial to ask: Should insects be afforded the same ethical consideration as vertebrates under the 3Rs framework? This essay explores the ethics of using insects in scientific research and how the 3Rs can or should apply to them. Drawing on ethical theories like utilitarianism, deontology, and virtue ethics, this paper argues that while insects do not evoke the same moral urgency as vertebrates, the ethical considerations inherent in the 3Rs should extend to insect research. This extension requires a reevaluation of the 3Rs, acknowledging the unique biological and cognitive characteristics of insects. By examining Replacement, Reduction, and Refinement in the context of insects, this essay contributes to a growing debate on how to responsibly and ethically conduct research involving invertebrate species.

2. Historical and Scientific Background

The 3Rs framework was first proposed in 1959 by William Russell and Rex Burch in their book The Principles of Humane Experimental Technique.[1] It was introduced as a guideline for ethical scientific research, emphasizing the need to respect and minimize harm to animals used in experiments. The principles of Replacement, Reduction, and Refinement were created to help researchers make decisions that would reduce the ethical cost of using animals in experiments.

Russell and Burch originally defined these principles as follows:

- Replacement: "Any scientific method employing non-sentient material (*sic*) which may in the history of animal experimentation replace methods with use conscious living vertebrates."
- Reduction: A means of minimizing, other than by replacement, "the number of animals used to obtain information of a given amount and precision."
- Refinement: Measures leading to "a decrease in the incidence or severity of inhumane procedures applied to those animals which have to be used."

From the definition alone, it is clear that insects (and other invertebrates) appear to be classified as non-sentient material, and based on the principle of replacement, they are suggested as alternatives to vertebrate animals. Indeed, the authors themselves mention that non-sentient material includes higher plants, microorganisms, and the more degenerate metazoan endoparasites, in which the nervous and sensory systems are almost atrophied, while free-living metazoan invertebrates (such as insects) were

[1] Russell WMS, Burch RL. The principles of humane experimental technique. Universities Federation for Animal Welfare, Wheathampstead (UK), 1959. (as reprinted 1992).

arbitrarily excluded from subjects of humanitarian concern:[2]

> A more difficult question arises when we consider the free-living metazoan invertebrates. We have arbitrarily excluded them from consideration as objects of humanitarian concern. It remains to consider them in the light of possible substitutes for vertebrate subjects. Such a procedure may be called comparative substitution.

However, this arbitrary exclusion is not related to the possibility that they might feel pain but primarily to the lack of research on similarities with humans.

3. Insects in Research

Most animal research involves insects or other invertebrates, as they offer numerous advantages over vertebrates, such as a relatively short life cycle, high reproductive rates, simple anatomy, and the ease with which large numbers of individuals can be studied.[3] Additionally, their use is significantly more economical, as thousands of invertebrates can easily be housed in a small laboratory.[4] The availability of large numbers of insects allows for statistically robust experiments and replicable studies, which are crucial for scientific validity. Research on invertebrates concerns either their direct impact on humans, such as the destruction of crops or the transmission of diseases, or their use as models for research related to genetics or physiology.

The use of invertebrates as models for human genetics and diseases in laboratory research dates to the late 19th century. Geneticist William E. Castle was one of the first to publish studies based on invertebrates: he used the ascidian *Ciona intestinalis* as a research model and published his dissertation on the species in 1896. Castle was also the first to use insects in his research: he conducted a long series of experiments (1901–1906) on inbreeding and outbreeding in the fruit fly *Drosophila melanogaster* (Diptera, Drosophilidae), and together with Charles Woodworth, he received recognition from Thomas H. Morgan, who was awarded the Nobel Prize in 1933 for his discoveries concerning the role of chromosomes in heredity. Since then, the fly *D. melanogaster* has been the most widely used animal in genetic studies. Apart from its ease of reproduction and cultivation, the molecular biology of this species is relatively simple, and a vast variety of mutant and genetically modified flies have been developed.[5] The genetics of flies has been crucial to the study of development, the cell cycle, ethology, and neuroscience. The similarities in the basic biochemistry of all animals allow us to use flies as simple models to investigate the

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[2] *idem.* p. 69.

[3] Jans K, Lüersen K, Rimbach G. *Drosophila melanogaster* as a Model Organism to Study Lithium and Boron Bioactivity. Int J Mol Sc 2021, 22:11710.

[4] Andre RG, Wirtz RA, Das YT. Insect Models for Biomedical Research. In: Woodhead AD (Ed) Non-mammalian Animal Models for Biomedical Research. CRC Press, Boca Raton, 1989:61-72.

[5] Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, Couto A, Marra V, Keleman K, Dickson, BJ. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature 2007, 448(7150):151-156.

genetics of various conditions, such as heart disease and neurodegenerative diseases.[6]

Apart from the fruit fly *D. melanogaster*, the greater wax moth *Galleria mellonella* (Lepidoptera, Pyralidae),[7] the silkworm *Bombyx mori* (Lepidoptera, Bombycidae),[8] and the red flour beetle *Tribolium castaneum* (Coleoptera, Tenebrioidae)[9] are used as laboratory animals in many studies. Other insects less frequently used as laboratory animals include the tobacco hornworm moth *Manduca sexta* (Lepidoptera, Sphingidae),[10] the seven-spotted ladybird *Coccinella septempunctata* (Coleoptera, Coccinellidae),[11] the yellow mealworm beetle *Tenebrio molitor* (Coleoptera, Tenebrionidae),[12] the scorpionfly *Panorpa cognata* (Mecoptera, Panorpidae),[13] as well as various species of mosquitoes and grasshoppers.[14] Their large numbers and the perception that they are less capable of suffering compared to vertebrates have made insects an ethically less controversial choice for research. However, this assumption raises important questions about their moral status and whether the 3Rs should apply equally to them.

4. Ethical Gaps in Considering Insects

Despite the growing use of insects in research, they are often excluded from the scope of most animal welfare regulations. The European Union, for instance, includes cephalopods like octopuses in its regulations for the protection of animals used for scientific purposes, but insects remain largely unprotected.[15] This gap presents an ethical challenge. While vertebrates are granted some form of moral consideration due to their capacity to suffer, insects are often excluded based on assumptions about their limited cognitive and emotional capabilities.

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[6] Marsh JL, Thompson, LM. Can flies help humans treat neurodegenerative diseases? BioEssays 2004, 26(5):485-96, Bier E, Bodmer R. *Drosophila*, an emerging model for cardiac disease. Gene 2004, 342(1):1-11.

[7] Mikulak E, Gliniewicz A, Przygodzka M, Solecka J. *Galleria mellonella* L. as model organism used in biomedical and other Studies. Przeglad Epidemiologiczny 2018, 72:57-73.

[8] Meng X. Zhu F, Chen K. Silkworm: a promising model organism in Life Science. J Insect Sci 2017, 17: 97, Abdelli N, Peng L, Keping C. Silkworm, *Bombyx mori*, as an alternative model organism in toxicological research. Environ Sci Pollut R 2018, 25:35048-35054.

[9] Rösner J, Wellmeyer B, Merzendorfer H. *Tribolium castaneum*: a model for investigating the mode of action of insecticides and mechanisms of resistance. Curr Pharm Des 2020, 26:3554-3568.

[10] Gershman A, Romer TG, Fan Y, Razaghi R, Smith WA, Timp W. *De novo* genome assembly of the tobacco hornworm moth (*Manduca sexta*). G3 Genes|Genomes|Genetics 2020, 11:jkaa047

[11] Ren XY, Zhang LS, Han YH, An T, Liu Y, Li YY, Chen HY. Proteomic research on diapause-related proteins in the female ladybird, *Coccinella septempunctata* L. Bull of Entom Res Lond 2016, 106:168-174.

[12] de Carvalho NM, Teixeira F, Silva S, Madureira AR, Pintado ME. Potential prebiotic activity of *Tenebrio molitor* insect flour using an optimized *in vitro* gut microbiota model. Food Funct 2019, 10:3909-3922.

[13] Engqvis, L, Sauer KP. Influence of nutrition on courtship and mating in the scorpionfly *Panorpa cognata* (Mecoptera, Insecta). Ethology 2003, 109:911-928.

[14] Smith RC. The mosquito as a laboratory animal. Am Biol Teach 1962, 24(7): 513-516, Badman J, Harrison J, McGarry M. Grasshoppers in research and education: methods for maintenance and production. Lab Anim 2007, 36:27-31.

[15] EU (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L276, 33-78.

However, recent research suggests that insects may experience pain and discomfort in ways that are more complex than previously thought. At least some insects have nociceptors—cells that detect and transmit signals responsible for the sensation of pain.[16] In a study by Tracey *et al.* on *Drosophila* larvae, researchers observed that the larvae responded to the touch of a heated probe with a stereotypical rolling behavior, which was different from their response to a non-heated probe.[17] Insects can detect and respond to harmful or disturbing stimuli, reacting in ways that protect their physical integrity. This ability is called nociception.[18] Unlike the conscious experience of pain, defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, nociception is an involuntary rapid reflex that lacks the emotional response or sensation associated with pain and does not require subjective reactions.[19] Thus, it is possible to have nociception without the conscious sensation of pain.

Despite the existence of nociception in insects, there is disagreement among scientists about whether insects can consciously feel pain. Eisemann *et al.* argue that it is impossible to provide a definite answer since an organism's subjective experience cannot be compared directly to that of another being.[20] To hypothesize about subjective experiences in other organisms, researchers must rely on experimental and theoretical criteria,[21] often using arguments by analogy.[22] For instance, if a mammal shows a particular behavior in response to a painful stimulus, such as an electric shock, and we conclude that it feels pain, we might draw the same conclusion if an insect reacts similarly—assuming we accept or reject the analogy for both.[23] The most relevant criteria for this comparison come from neurophysiology and ethology.

There are several neurophysiological and behavioral signs that suggest invertebrates, and potentially insects, might feel pain. One such indication is the presence of natural opioids and analgesics in their nervous system. The presence of endogenous opiates in animals is strong evidence that they experience pain.[24] Natural opioids help regulate pain to reduce its intensity.[25]

[16] Eisemann CH, Jorgensen WK, Merritt DJ, Rice MJ, Cribb BW, Webb PD, Zalucki MP. Do insects feel pain? - A biological view. Experientia 1984, 40:164-167.
[17] Tracey J, Wilson RI, Laurent G, Benzer S. painless, a *Drosophila* gene essential for nociception. Cell 2003, 113(2):261-273.
[18] Kavaliers M. Evolutionary and comparative aspects of nociception. Brain Res Bull 1988, 21: 923-931, Smith JA. A question of pain in insects. ILAR J 1991, 33(1-2):25-32.
[19] Bateson P. Assessment of pain in animals. Anim Behav 1991, 42:827-839, Broom DM. Evolution of pain. In: Soulsby EJL, Morton D (eds.), Pain: Its Nature and Management in Man and Animals. Royal Society of Medicine International Congress Symposium Series, vol 246. Royal Society of Medicine, London 2001:17-25, Mather JA. Animal suffering: An invertebrate perspective. J Appl Anim Welf Sci 2001, 4:151-156.

[20] Eisemann, *op. cit.*
[21] Dennett D. Consciousness Explained. Little, Brown and Company, Boston 1991.
[22] Sherwin CM. Can Invertebrates Suffer? Or, How Robust is Argumentby-analogy? Anim Welf 2001, 10(supplement 1):103-118.
[23] Elwood RW, Barr S, Patterson L. Pain and Stress in Crustaceans? Appl Anim Behav Sci 2009, 118:128-136.
[24] Rollin BE. The Unheeded Cry: Animal Consciousness, Animal Pain, and Science. Iowa State University, Ames 1998:154.
[25] Elwood, *op. cit.*

Therefore, a question arises on whether insects have a moral status that justifies applying the 3Rs framework. The failure to address this issue reflects a bias in ethical thinking that favors animals closer to humans on the evolutionary scale, while potentially overlooking the ethical treatment of species that are more distantly related.

5. Philosophical Perspectives

5.1. Utilitarianism and Insect Research

Utilitarianism, a consequentialist theory founded by Jeremy Bentham and John Stuart Mill, holds that the rightness of an action is determined by its outcomes, specifically by the amount of pleasure or pain it generates. The use of insects in research is typically justified from a utilitarian perspective on the grounds that the benefits of scientific knowledge far outweigh the harm caused to the insects. However, utilitarianism also requires that we consider the suffering of all sentient beings, no matter how small. The question, then, is whether insects are capable of suffering to an extent that would make their use in research ethically problematic. If insects can suffer, even minimally, utilitarian ethics would demand that we reduce or refine their use in research to minimize harm.

At the same time, the benefits of using insects in research—such as advances in medicine, agriculture, and environmental science—are significant. For instance, research on *Drosophila* has led to breakthroughs in understanding genetic diseases.[26] A utilitarian might argue that the relatively small amount of harm inflicted on insects is justified by these substantial benefits to human and animal health.

5.2. Deontological Ethics and the Moral Duty Toward Insects

In contrast to utilitarianism, deontological ethics, particularly as articulated by Immanuel Kant, is concerned with the moral duties we have, independent of the consequences. Deontology holds that certain actions are inherently right or wrong, based on principles or rules, rather than outcomes. From this perspective, we might ask whether humans have a moral duty toward insects in research, regardless of the benefits that such research might produce.

Kant's categorical imperative suggests that we should treat all rational beings as ends in themselves, not merely as means to an end. Since insects are not rational beings by Kant's definition, one could argue that they fall outside the scope of this duty. However, the challenge here is whether rationality is the only criterion for moral consideration. Modern deontological perspectives have expanded Kantian ethics to include non-rational beings, arguing that moral duties extend beyond rational agents.[27] Some scholars propose that animals, including insects, should not be used merely as tools for human benefit if it leads to unnecessary suffering.[28]

[26] Bonini NM. A perspective on *Drosophila* genetics and its insight into human neurodegenerative disease. Front Mol Biosci 2022, 9:1060796.

[27] Wood AW, O'Neill O. Kant on Duties Regarding Non-rational Nature. Proc Aristot Soc Suppl Vol 1998, 72:189-228.

[28] Denis L. Kant's Conception of Duties Regarding Animals: Reconstruction and Reconsideration. Hist Philos Q 2000, 17:405-423, Camenzind S. Kantian Ethics and the Animal Turn. On the Contemporary Defence of Kant's Indirect Duty View. Animals 2021, 11:512.

This view might advocate for the application of the 3Rs, especially Refinement, even if insects are not rational agents. By refining experimental procedures, researchers could minimize harm and fulfill a moral duty to treat even non-rational beings with a certain level of respect. Therefore, from a deontological perspective, we could argue that humans have a duty to incorporate the 3Rs in research involving insects—not because insects have rights *per se*, but because it aligns with broader moral duties to avoid cruelty and respect the natural world.[29]

5.3. Virtue Ethics and the Moral Character of Researchers

Virtue ethics, derived from the philosophy of Aristotle, shifts the focus from rules and consequences to the character of the individual acting. According to virtue ethics, moral behavior is that which cultivates virtues such as compassion, temperance, and wisdom. The ethical use of animals in research, including insects, depends not just on the outcomes of research or abstract moral duties, but on the virtues or vices that researchers exhibit in their treatment of these creatures.

A researcher who embodies the virtue of compassion might question whether causing harm to insects in research is truly necessary, even if the insects' capacity for suffering is minimal. Compassionate researchers would be motivated to apply the 3Rs as a matter of moral character, not just because of external rules or consequences, but because they believe it is the right thing to do. They would seek to refine methods to avoid unnecessary harm and would be open to replacing insect models with non-animal alternatives where possible.

At the same time, a virtue ethicist would likely recognize that the role of research in improving human and animal welfare is itself virtuous.[30] The pursuit of knowledge and innovation, when done responsibly, contributes to the flourishing of humanity and other species. Therefore, a researcher who uses insects in a manner consistent with the 3Rs could be seen as balancing virtues—compassion for the insects and responsibility toward the broader human and ecological community.

In this sense, virtue ethics provides a holistic approach to the ethical challenges posed by insect research. It encourages researchers to reflect on their own moral character and to strive for excellence not just in scientific rigor, but in ethical sensitivity as well.

6. Applying the 3Rs to Insect Research

Having explored the philosophical frameworks, we turn to how the 3Rs can and should be applied to research involving insects. Each of the 3Rs presents unique challenges when considered in relation to insects, particularly given the differences in cognitive capacity and sentience between insects and vertebrates.

6.1. Replacement

The principle of Replacement encourages researchers to use non-animal models whenever possible. In the case of insect research, this principle raises the question of whether insects

[29] Camenzind, *op. cit.*

[30] Hursthouse R. Virtue Ethics and the Treatment of Animals. In: Beauchamp TL, Frey RG (eds.), The Oxford Handbook of Animal Ethics. Oxford Handbooks 2011; online edn, Oxford Academic.

can be replaced by non-sentient alternatives such as computer models, simulations, or *in vitro* systems. These alternatives not only address ethical concerns but also offer the potential for more refined and accurate scientific outcomes. However, while computer models and other non-animal methods are improving, they have not yet reached the level of complexity necessary to fully replace insects in the fields of genetic research, toxicology, and behavioral studies.

One of the most promising alternatives to using live insects in research is the development of *in silico* methods, which rely on computer simulations and mathematical models to study biological processes.[31] These methods offer several advantages, like predictive modeling based on existing data, simulating molecular and genetic processes at a high level of detail, but they also allow researchers to integrate and analyze large datasets from multiple sources, including genomic, transcriptomic, and proteomic data. Examples of *in silico* methods and computational models as alternatives to insects include *in silico* toxicology models,[32] computational models of insect nervous systems,[33] virtual fly models of *Drosophila* simulators for genetics research,[34] and metabolic and physiological modeling.[35] By reducing the reliance on live insects, these methods contribute to the ethical goal of minimizing harm to living beings. Despite their potential, *in silico* methods are not without limitations. Computational models are only as good as the data they are based on, and inaccuracies in the data can lead to flawed predictions. Moreover, some biological processes are too complex to be fully captured by current modeling techniques. Hence, while *in silico* methods offer a valuable alternative to insect research, they are often used in conjunction with *in vivo* experiments rather than as a complete replacement.

In vitro techniques involve studying biological processes outside of a living organism, typically in a controlled laboratory environment using cells, tissues, or biochemical systems. These techniques serve as ethical alternatives to *in vivo* studies in insects, adhering to the replacement principle. Examples include cell culture systems for studying various biological processes such as gene expression, metabolism, signal transduction, and viral replication,[36] tissue engineering and organotypic cultures, which are three-dimensional tissue models that closely replicate the structure and

[31] Madden JC, Enoch SJ, Paini A, Cronin MTD. A Review of *In Silico* Tools as Alternatives to Animal Testing: Principles, Resources and Applications. Altern Lab Anim 2020, 48(4):146-172.
[32] *ibidem.*
[33] Mosqueiro TS, Huerta R. Computational models to understand decision making and pattern recognition in the insect brain. Curr Opin Insec Sci 2014, 6:80-85.
[34] Cresiski RH. Two Virtual Labs to Study Genetic Inheritance in the Fruit Fly. J Microbiol Biol Educ 2013, 14(1):141-142.
[35] Hall RJ, Thorpe S, Thomas GH, Wood AJ. Simulating the evolutionary trajectories of metabolic pathways for insect symbionts in the genus *Sodalis*. Microb Genom 2020, 6(7):mgen000378, Cesur MF, Basile A, Patil KR, Çakır T. A new metabolic model of *Drosophila melanogaster* and the integrative analysis of Parkinson's disease. Life Sci Alliance 2023, 6(8):e202201695.
[36] Schneider I. Cell lines derived from late embryonic stages of *Drosophila melanogaster*. J Embryol Exp Morphol 1972, 27(2):353-365, He X, Lu L, Huang P, Yu B, Peng L, Zou L, Ren Y. Insect Cell-Based Models: Cell Line Establishment and Application in Insecticide Screening and Toxicology Research. Insects 2023,14(2):104.

function of whole organs,[37] and high-throughput screening (HTS), which allows researchers to rapidly test large numbers of chemical compounds or genetic modifications for their effects on insect cells or tissues, especially in the fields of drug discovery, pesticide development, and genetic research.[38] While *in vitro* techniques offer significant ethical and practical advantages, they also have limitations. The complexity of whole-organism interactions cannot always be replicated *in vitro*, and some physiological processes may require the context of a complete, living system to be fully understood.[39] Nevertheless, *in vitro* methods represent a valuable alternative to insect research, particularly in the early stages of scientific inquiry.

Another promising alternative to insect research is the use of non-animal models and biomimetics, where biological principles observed in insects are replicated using artificial or synthetic systems. Examples include the use of Artificial Neural Networks (ANNs) which can be used to simulate insect behavior, decision-making, and learning processes, like the navigational strategies of bees and the foraging behavior of ants,[40] robotics and biomimetics systems,[41] and synthetic biology and biohybrid systems, like biohybrid drones and engineered tissues.[42] Non-animal models and biomimetics offer a compelling alternative to traditional insect research. However, these approaches also have limitations, as they may not fully capture the complexity of living organisms. Nevertheless, they represent an innovative and ethically sound approach to studying insect-related phenomena.

6.2. Reduction

Reduction focuses on minimizing the number of animals used in research while still obtaining valid results. Insects, because of their small size and short life cycles, are often used in large numbers. Entire populations of insects can be easily manipulated or destroyed in a single experiment, raising questions about whether the principle of Reduction is being sufficiently applied. Despite their numerical abundance, applying Reduction to insect research is an ethical necessity, ensuring that experiments do not use more individuals than required while maintaining scientific integrity.

[37] Napoleão TH, Albuquerque LP, Santos ND, Nova IC, Lima TA, Paiva PM, Pontual EV. Insect midgut structures and molecules as targets of plant-derived protease inhibitors and lectins. Pest Manag Sci 2019, 75(5):1212-1222.

[38] Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD, Kidd MJ, King AM, Meyer MR, Slade D, Lum PY, Stepaniants SB, Shoemaker DD, Gachotte D, Chakraburtty K, Simon J, Bard M, Friend SH. Functional discovery via a compendium of expression profiles. Cell 2000,102(1):109-26.

[39] Forestiero S. The historical nature of biological complexity and the ineffectiveness of the mathematical approach to it. Theory Biosci. 2022, 141(2):213-231.

[40] Knaden M, Graham P. The Sensory Ecology of Ant Navigation: From Natural Environments to Neural Mechanisms. Annu Rev Entomol 2016, 61(1):63-76.

[41] Fry SN. Experimental Approaches Toward a Functional Understanding of Insect Flight Control. In: Floreano D, Zufferey JC, Srinivasan MV, Ellington C (eds.) Flying Insects and Robots. Springer, Heidelberg Dordrecht London New York 2010:1-14.

[42] Lentink D, Dickinson MH. Biofluid dynamic scaling of flapping, spinning and translating fins and wings. J Exp Biol 2009, 212(16):2691-2704, Webster-Wood VA, Guix M, Xu NW, Behkam B, Sato H, Sarkar D, Sanchez S, Shimizu M, Parker KK. Biohybrid robots: recent progress, challenges, and perspectives. Bioinspir Biomim 2022, 18:015001.

To ethically apply Reduction to insect research, scientists must balance the need for large sample sizes with the ethical imperative to minimize harm. This might involve developing more precise statistical methods to reduce the number of insects used in experiments or improving experimental designs to gather the necessary data with fewer subjects. To adhere to the principle of Reduction, scientists must employ advanced statistical methods and experimental designs that allow them to achieve valid results with fewer subjects. For instance, power analysis, a statistical technique used to determine the minimum sample size required to detect an effect with a given degree of confidence,[43] could be used in insect research. This method can significantly reduce the number of insects used by ensuring that sample sizes are neither too large nor too small to produce meaningful results.

Another effective approach in Reduction is the use of pilot studies, which can provide critical preliminary data to guide full-scale experimental designs. Pilot studies allow researchers to estimate effect sizes, identify potential sources of variability, and optimize protocols before committing to large-scale trials.[44] By conducting small-scale preliminary experiments, researchers can refine their hypotheses, select the most effective methodologies, and determine the necessary sample sizes more accurately. This not only reduces unnecessary insect use but also enhances the overall efficiency of research, reducing costs and time investment. Recent discussions in the literature have underscored the importance of pilot studies in determining appropriate sample sizes and improving experimental design in animal research.[45]

At the same time, it is important to recognize that the biological characteristics of insects—such as their high reproductive rates—complicate the application of Reduction. Unlike vertebrates, whose individual lives might hold more significance from a moral perspective, insects are often viewed as part of a collective group. Nevertheless, ethical research demands that we apply Reduction wherever possible, even to species that reproduce quickly and exist in large numbers.

6.3. Refinement

Refinement seeks to minimize suffering and improve the welfare of animals used in research. In vertebrate studies, this principle is applied through better living conditions, the use of anesthesia or analgesia to reduce pain, and the implementation of humane endpoints in experiments. The ethical considerations surrounding insect research have traditionally been overlooked, but emerging evidence suggests that insects may have more complex responses to harmful stimuli than previously assumed.[46] This underscores the necessity of incorporating Refinement into insect research by

[43] Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev 2007, 82(4):591-605.

[44] Teare MD, Dimairo M, Shephard N, Hayman A, Whitehead A, Walters SJ. Sample size requirements to estimate key design parameters from external pilot randomised controlled trials: a simulation study. Trials 2014, 5:264.

[45] Laws TR, Maishman TC. Considerations in the design of animal infection pilot studies. Front Cell Infect Microbiol 2022, 12:948464.

[46] Crump A, Gibbons M, Barrett M, Birch J, Chittka L. Is it time for insect researchers to consider their subjects' welfare? PLoS Biol 2023, 1;21(6):e3002138.

improving handling techniques, using appropriate anesthesia, and ensuring humane euthanasia where necessary.

Anesthesia is deemed essential for procedures requiring immobilization, such as microscopic examination and sampling, as well as for procedures that may cause pain or distress, including surgical interventions, electrophysiological studies, and magnetic resonance imaging (MRI).[47] It is important for researchers and technicians working with insects to be familiar with and able to use appropriate anesthesia techniques for each species. There is sufficient research and a variety of methods available for anesthetizing insects.[48] Carbon dioxide (CO_2) is the most popular agent to immobilize insects in entomological research, although multiple side effects and high mortality makes its use controversial, and a more progressive approach would be the use of a volatile anesthetic agent like isoflurane or sevoflurane.[49] However, species-specific responses must be considered, as different insects exhibit varying levels of resistance to hypoxia and chemical agents. For example, cockroaches (Blattodea) show remarkable tolerance to hypoxia and can survive prolonged oxygen deprivation.[50]

Since little is known about the needs of different species, understanding the physiology of each species is essential, such as maintaining fluid balance, as with all animal species.[51] Insects are small, with a large surface area relative to their body mass, making them prone to dehydration. The use of analgesics in invertebrates does not yet appear to be feasible; since they do not possess a central nervous system with a well-described cortex or similar structure, it is not clear whether they perceive pain and suffer emotional distress from it. For instance, although snails exhibit withdrawal and escape behaviors in response to mechanical, chemical, and electrical stimuli, the presence of an analgesic appears to diminish or slow these responses.[52] However, it remains uncertain whether this reduction is attributable to a sedative effect or the analgesic properties of the drug.[53] As an alternative, anesthesia is recommended for any procedures that may be painful or disrupt the insect's normal behavior.[54]

The practice of euthanasia in laboratory insects is not widely represented in existing protocols. With the exception of the UFAW handbook series, which has included information on invertebrate anesthesia and euthanasia since

[47] Cooper JE. Anesthesia, analgesia, and euthanasia of invertebrates. ILAR J 2011, 52(2):196-204.

[48] Lewbart GA (ed.) Invertebrate Medicine. (3rd ed). John Wiley & Sons inc, Hoboken 2022.

[49] Wahltinez SJ, Harms CA, Lewbart GA. Chapter 26 - Anesthesia and analgesia in invertebrates, In: Dyson MC, Jirkof P, Lofgren J, Nunamaker EA, Pang D (eds.) Anesthesia and Analgesia in Laboratory Animals (Third Edition), Academic Press, Cambridge 2023:647-671.

[50] Natalie G. Schimpf, Philip G. D. Matthews, Craig R. White, Cockroaches that exchange respiratory gases discontinuously survive food and water restriction, Evolution 2012, 66(2):597-604.

[51] Kirby R, Rudloff E. Fluid balance. In: Kirby R, Linklater A. (eds), Monitoring and Intervention for the Critically Ill Small Animal: The Rule of 20. John Wiley & Sons inc, Hoboken 2016:9-28.

[52] Kavaliers M, Hirst M. Tolerance to morphine-induced thermalresponse in terrestrial snail, *Cepaea nemoralis*. Neuropharmaology 1983, 22: 1321e1326, Kavaliers M, Hirst M, Teskey GC. A functional role for an opiate system in snail thermal behavior. Science 220 (4592):99e101.

[53] Wahltinez *et al.*, *op. cit.*

[54] Cooper, *op. cit.*

1967, including methods for the "*tranquilizing and killing insects and ticks*",[55] there are no specific guidelines. In the literature, extensive references to euthanasia methods are made by Lewbart, but most of these have not been sufficiently studied.[56] Bennie *et al.* proposed injection sites and doses for different orders of arthropods,[57] extending the Potassium chloride (KCl) technique of Battison *et al.* for euthanasia of the American lobster (*Homarus americanus*),[58] but this method is hard to use for small insects. However, information is available on certain characteristics of ectothermic animals (mainly reptiles and amphibians), such as severing the nervous tissue, which may relate to some invertebrates.[59] Other euthanasia methods that can be used are rapid decapitation - although insects have different nervous systems from vertebrates and decapitation alone may not always be sufficient to destroy neural functions[60] - and immersion in ethanol. For the euthanasia of arachnids, which are closely relatives to insects, Pizzi and Kennedy recommended immersion in 70% ethanol, dismissing the method of rapid freezing for these and other invertebrates, as the resulting tissue damage risks compromising histological examination.[61] In any case, euthanasia methods for insects have been insufficiently researched and require further investigation.

Another important issue that it has to be under Refinement is insects' welfare. Due to indications that insects may be capable of experiencing sensations, the precautionary principle should be invoked when designing legislation for the welfare and protection of insects, and it should be applied across the board. The precautionary principle was defined by Birch:[62]

> When there are threats of serious negative outcomes for the welfare of animals, the lack of complete scientific certainty regarding the sentience of those animals is not used as a reason to postpone economically feasible measures to prevent those outcomes.

Furthermore, legislation must be enacted to ensure the well-being of insects used in research. Good conditions relate to the housing and environmental conditions, their nutrition, and anything else that might affect their well-being. The questions that arise, of course, pertain to the very nature of insect living conditions and what might constitute 'well-being' for them. The lack of clarity regarding the presence of emotional

[55] UFAW. The UFAW Handbook on the Care and Management of Laboratory Animals, 3rd ed. Section IV: Birds, Poikilotherms and Invertebrates. E&S Livingstone, Edinburgh and London, 1967.

[56] Lewbart, *op. cit.*

[57] Bennie NAC, Loaring CD, Bennie MMG, Trim SA. An effective method for terrestrial arthropod euthanasia. J Exp Biol 2012, 215(24):4237-4241.

[58] Battison A, MacMillan R, MacKenzie A, Rose P, Cawthorn R, Horney B. Use of injectable potassium chloride for euthanasia of American lobsters (*Homarus americanus*). Comp Med 2000, 50(5):545-50.

[59] Cooper JE, Ewbank R, Platt C, Warwick C. Euthanasia of Amphibians and Reptiles. Report of a Joint UFAW/WSPA Working Party. Universities Federation for Animal Welfare, Potters Bar, 1989.

[60] Gunkel C, Lewbart GA. "13. Invertebrates". In West G, Heard D, Caulkett N (eds.). Zoo Animal & Wildlife Immobilization and Anesthesia. Blackwell, Oxford, 2007:147-158.

[61] Pizzi R, Kennedy B. Spiders. In: Lewbart GA (ed.). Invertebrate Medicine. (3rd ed.). John Wiley & Sons inc, Hoboken, 2022:301-348.

[62] Birch J. Animal sentience and the precautionary principle. Anim Sentience 2017, 16(1):1-15.

states in insects makes it difficult to know what actions should be taken to optimize their living conditions.[63] The International Platform of Insects for Food and Feed (IPIFF) suggests adopting Brambell's Five Freedoms as a basis for establishing good practices and proper treatment of farmed insects, provided these take into account the specific characteristics of insects.[64] Five Freedoms, as derive from Brambell's quote,[65] are: freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom to express normal behavior, and freedom from fear and distress.

Although the welfare of insects has not traditionally been a priority in research, applying Refinement to insect studies would represent an important ethical step forward.

7. Conclusion

Insects are increasingly used in scientific research, yet their moral and ethical status remains a matter of debate. Through the lens of the 3Rs—Replacement, Reduction, and Refinement—this essay has explored the ethical complexities of using insects in research, drawing on utilitarian, deontological, and virtue ethics perspectives. While insects may not possess the same cognitive and emotional capacities as vertebrates, emerging evidence suggests that they are capable of more complex experiences than previously assumed.

The ethical application of the 3Rs to insect research requires a nuanced understanding of both the scientific and philosophical dimensions of the issue. The 3Rs should extend to insects, even if the practical challenges differ from those faced in vertebrate research. By continuing to refine our ethical frameworks and scientific practices, we can ensure that research involving insects is conducted responsibly and humanely, in a manner that respects both the needs of science and the ethical imperative to reduce harm.

[63] Barron AB, Klein C. What insects can tell us about the origins of consciousness. Proc Natl Acad Sci 2016, 113(18):4900-4908.

[64] IPIFF, (2019). Ensuring High Standards of Animal Welfare in Insect Production. https://ipiff.org/wp-content/uploads/2019/02/Animal-Welfare-in-Insect-Production.pdf

[65] Brambell R. Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems. Her Majesty's Stationary Office, London, 1965:13.