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On the origin of 'bloopergenes': unraveling the evolution of the balanced lethal system in *Triturus newts*

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Chapter 7 - General discussion

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This Chapter concludes my genomic studies of chromosome 1 syndrome in *Triturus*. The studies conducted at the Wielstra lab are part of the first research project that uses genomic information of *Triturus* newts with the aim of unraveling the evolution of balanced lethal systems in nature. Furthermore, the insights gained, as well as the methodologies and tools developed in the process, have a broader utility and thus a wider scientific impact. Therefore, I divide this Chapter into two parts. In the first part, I briefly discuss the new insights into the evolution of the balanced lethal system in *Triturus* provided by this dissertation, and I suggest avenues for further research. In the second part, I discuss the broader ways in which this dissertation contributes to science (and science education).

Part I – Balanced Lethal Systems and Further Research

For years on end, my colleagues and I have hypothesized that a balanced lethal system such as chromosome 1 syndrome in *Triturus* consists of two supergene versions with unique, recessive lethal alleles [1, 2] that;

- 1) Originated via a slow ‘degradation process’, driven by heterozygote advantage, balancing selection, and mutation accumulation due to a lack of recombination (see **Chapters 1, 2 and 6**), and/or;
- 2) Became established as one of the two versions – both uniquely involved in different species – entered an ancestral genome via introgressive hybridization, which facilitated fixation in the population (see **Chapters 1 and 6**).

Both of these theories are broadly applicable to diploid organisms, with the first (i.e. the slow degradation process for two supergene variants that got caught in a balanced polymorphism) gaining attention in recent population modeling studies [3, 4].

Much to our surprise, the evidence uncovered in my research does not support this gradual degradation model, but instead pointed toward a rapid – practically instantaneous – origin of the balanced lethal system in *Triturus* [see Chapter 6 and; 5]. This idea had already been suggested by researchers over 35 years ago: “*We postulate that the chromosome 1 arrest syndrome is the result of a cytogenetic accident involving an unequal genic exchange between the homologues of chromosome 1 in the common ancestor ... Such an exchange could potentially result in the formation of a balanced lethal chromosomal heteromorphism in one step if the exchange: (1) was large enough to abolish homologous pairing and crossing over in the exchanged regions, (2) took place in the germ line, and (3) the exchanged material included DNA sequences that were important in early embryonic development*” [quoted from; 6]. However, these researchers never had the empirical evidence to support this claim – something that I have now co-produced.

Furthermore, with the second theory (i.e. introgressive hybridization brought both chromosomes, 1A and 1B, together in one ancestral genome) I expected to find that only the 1A-linked genes, or only the 1B-linked genes, would show evidence of a distinct evolutionary history. Instead, we not only found this for both sets of genes, but also for chromosome 1 as a whole (i.e., also including the colinear recombining region), complicating the evolutionary narrative even further (see **Chapter 6**). While we have demonstrated that introgressive hybridization likely played a role in the aberrant evolution of chromosome 1 overall, the extent of its involvement in the formation of the balanced lethal system remains unclear.

In short, this dissertation thus offers some of the first, big puzzle pieces necessary for understanding the evolutionary origin of the balanced lethal system that is chromosome 1 syndrome. Also, **Chapter 6** of this dissertation introduces the term ‘bloopergene’ into the scientific literature – highlighting that, despite their name, supergenes do not always result in ‘superlative’ outcomes for a species. However, this dissertation also demonstrates that scientific inquiry is an ongoing process, and that in relation to the origin of balanced lethal systems – both in general, as well as for the case in *Triturus* specifically – many unanswered questions remain. These can be solved in the future by applying more (elaborate) techniques.

Follow-up research: Whole Genome Studies

Firstly, while the ‘NewtCap’ target capture methodology (see **Chapter 4**) provides a large amount of highly insightful data, it only captures a part of the coding regions of the DNA of *Triturus*. As a result, we so far still lack a comprehensive list of genes that show (presence/absence) variation associated with the balanced lethal system for each of the affected species. Secondly, while the structural variation and gene order we hypothesize for chromosomes 1A and 1B are likely accurate given the high synteny among species used in the comparative genomic analysis [see the ancestral constitution posed in **Chapter 6** and see; 5], it is worth noting that this remains an assumption at this point. This means that our ‘next-generation sequencing’ techniques have proven useful in providing us with certain ‘pieces of the puzzle’, however it also means that the next step would be to obtain *all* the pieces, i.e. to sequence the entire genome. This can be done by performing so-called ‘third-generation sequencing’ techniques [7].

Future studies utilizing third-generation sequencing techniques such as whole genome sequencing of long reads – something that is currently in progress at the Wielstra lab – will help clarify most issues. I anticipate that these studies will confirm the findings presented in this dissertation. Ideally, third-generation-based whole genome sequencing will soon yield a high quality, chromosome-level reference assembly for *Triturus*, in order to further support re-sequencing projects (which could include both third-generation-

based whole genome sequencing approaches, as well as next-generation-based whole genome sequencing approaches). Once this is achieved, whole genome sequencing studies could provide a much more accurate and complete picture of *Triturus* genomes for each species, making a more detailed ancestral reconstruction of chromosome 1 possible, which in turn would help explain the evolutionary origin of it. The genotyping methods introduced in **Chapter 3** will likely be a valuable resource to support the upstream laboratory work required for executing such future whole genome sequencing projects efficiently and at the lowest costs possible.

Follow-up research: Transcriptomics and Embryology

While this dissertation does not extensively focus on the specific causes of developmental arrest in 50% of the *Triturus* embryos, it is worth mentioning that our work opens new research avenues to delve deeper into the biology of the disease itself. As described in **Chapter 1**, embryonic arrest occurs during the phylotypic stage – a period that is highly conserved in terms of both morphology and underlying, regulatory pathways. This suggests that the candidate lethal genes involved in the disrupted embryonic development could belong to the so-called ‘evo-devo gene toolkits’ [8] – genes that are required for basic, embryonic modules or their regulation, such as the Hox genes that determine the body plan organization. Alternatively, the candidate lethal genes could also simply be essential ‘house-keeping’ genes, for instance [9].

Although my co-workers did try to analyze the main functionality of the 1A- and 1B-linked targets through a gene ontology analysis [5], no pattern so far stands out. Thus, it remains to be determined precisely what role the 1A- and 1B-linked genes play in causing chromosome 1 syndrome, as well as how they interact with each other and with other genes, in terms of cellular components, molecular functions, and biological processes.

Currently, researchers of the Wielstra lab have started conducting transcriptomic studies to examine the gene expression patterns at different embryonic stages that lead up to the deadly late tail-bud stage per each embryonic class (healthy 1A1B/1B1A, diseased 1A1A, or diseased 1B1B). These studies do not only aim to compare the expression patterns between 1A- and 1B-linked genes (as essentially the genes responsible for lethality should differ between the two, a prerequisite of a balanced lethal system as also shown by my results in this dissertation), but also across all *Triturus* species. Both studies focusing on RNA, as well as embryological studies focused on morphology, will help pinpoint the specific genes and molecular pathways involved in the *Triturus* balanced lethal system, adding an ‘evo-devo’ perspective on the subject at hand.

With such future studies, it is important to always keep in mind that ever since the balanced lethal system became fixed in the ancestor of *Triturus*, natural selection would not have been able to eliminate new harmful (including lethal) alleles that cause earlier arrest (as the fitness cannot drop below zero – which is why I referred to the balanced lethal system as the ultimate evolutionary failure in **Chapter 1**). Given this, there likely are differences between the species – although we now have reason to believe that the effect of this ‘ongoing decay’ has, surprisingly, been relatively minor (see **Chapter 6**). As with whole genome sequencing studies in the future, I foresee that for transcriptomic and embryological testing particularly **Chapter 3** (on classifying embryo’s in the laboratory) will prove helpful.

Part II – The Broader Impact of this Dissertation

Apart from delivering new insights into the evolutionary origin of the *Triturus* balanced lethal – an inherently compelling topic – this dissertation holds a broader relevance in both science and education. Namely, I believe that understanding the evolution of supergenes and bloopergenes in nature is not only fundamentally important, but can also indirectly have more practical implications. Here, I am not referring to stopping, or reversing, the balanced lethal system in *Triturus* embryos – after all, their ancestors thrived and speciated despite their evolutionary constraints. Rather, the insights gained from studying this system can be useful for understanding and addressing various other natural phenomena that share some of the key features of balanced lethal systems, particularly in fields where managing genetic diversity, or mitigating the effects of harmful alleles, is more relevant. Thus, I highlight some noteworthy parallels below. Finally, I will expand the scope even further by discussing how the methods and tools developed for this dissertation have the potential to advance future research projects, especially studies related to genomics and biodiversity (with a focus on salamanders).

From sickle-cell anemia in humans to hybrid vigor in maize

A balanced lethal system represents the most extreme form of heterozygote advantage possible, as all homozygotes inevitably die before reaching a reproductive age. However, more cases exist where heterozygotes that carry certain dominant alleles alongside disease-causing, recessive alleles, have a higher fitness compared to both types of homozygotes that carrying two similar copies of those harmful alleles. Thus, understanding the evolutionary mechanisms that allow harmful alleles to evolve and persist in populations could deepen our understanding of balancing selection in other contexts as well.

Sickle-cell anemia poses an example. On the one hand, humans that are homozygous for the ‘mutated’ hemoglobin allele (HbS/HbS) suffer from sickle-cell anemia: a condition in which red blood cells deform, leading to serious health problems and sometimes death [10-12]. On the other hand, if a person is homozygous for the ‘normal’ hemoglobin allele (HbA/HbA) they will not develop this heritable disease – however they are fully susceptible to severe malaria, caused by *Plasmodium falciparum* [13]. Intriguingly, individuals that are heterozygous for the sickle-cell-associated trait (HbA/HbS), and that thus carry one normal and one mutated hemoglobin allele, are protected against the malaria parasite *without* developing the severe symptoms of sickle-cell anemia [14-16]. Thus, balancing selection maintains the harmful hemoglobin allele in human populations that occur in areas with a high prevalence of malaria due to heterozygote advantage, as both types of homozygotes are less fit [15, 17, 18]. In this case, the outcome is of course heavily influenced by environmental factors – which is not applicable (anymore) to balanced lethal systems – but the driving, evolutionary mechanisms are similar nonetheless. Essentially, this example of balancing selection in humans demonstrates the same thing the *Triturus* balanced lethal system teaches: nature does not always offer an ‘easy way out’, as natural selection is never able to anticipate future conditions.

Another relevant context is that of plant cultivation practices in agricultural science. For instance, hybrid vigor (another word for heterozygote advantage) can be used to improve crops, such as corn [19]. When two different, homozygous (inbred) lines are crossed, this can result in heterozygous (hybrid) offspring that outperform the parent lines in terms of fitness. This can lead to enhanced yield and disease resistance, for example [20]. In fact, the word ‘Hi-Bred’ that later became the more general word ‘hybrid’ was first used by a company in 1926 in the context of crop improvement by applying hybrid vigor through crossing different breeds [21]. For corn specifically, hybrid breeds can show a yield increase of 30% compared to non-hybrid varieties [22] and they are more resistant to diseases like stalk rot [23] and leaf blight [24]. Also, corn hybrids may be more equipped to deal with external stressors, such as drought conditions [25] – something that is of importance in regions affected by climate change or inconsistent rainfall patterns. Thus, by understanding how balanced lethal systems evolve naturally, insights can be obtained that can in turn be used to study and improve hybrid vigor artificially. This will lead to higher food security and increased sustainability – which are of economic and environmental importance.

A helicopter view

My research primarily addresses a fundamental question within evolutionary biology. But no research is ever truly fundamental, as methods and tools developed for fundamental research often find applications in unrelated fields. This dissertation is an example of that:

combined, **all Chapters** form a coherent story describing all the chronological steps that I took over the course of five years to study the *Triturus*' balanced lethal system with available molecular techniques. But most of the work conducted holds potential for applications beyond this original research scope as well. For the final part of this Chapter, I would thus like to zoom out to overlook certain elements that have been briefly touched upon in other parts of this dissertation – and while doing so I will elaborate on the three main topics that personally drive me as an applied scientist.

Animal Welfare

“The least I can do is speak out for those who cannot speak for themselves” – Dr. Jane Goodall

Surprisingly, this dissertation could offer an innovative solution to some pressing animal welfare concerns in the intensive farming industry. This topic relates specifically to **Chapter 3**, where we discuss the potential of our mxKASP method for sexing embryos while still inside the egg (also referred to as ‘*in ovo* sex determination’). Worldwide, this practice is investigated in order to address ethical and economic issues, particularly in the context of male chick culling in the poultry industry. While our mxKASP approach was originally developed to differentiate between the genotypes of diseased and healthy *Triturus* embryos (see **Chapter 3**), it can certainly be adapted for broader use. Although implementing mxKASP to genotype chicken embryos at embryonic stages in which pain perception is physiologically implausible at a large scale would involve numerous steps, it is crucial to realize the importance of cultivating novel ideas that can sometimes emerge from unrelated research efforts.

Annually, it is estimated that seven *billion* one-day-old male chicks are slaughtered in the poultry industry worldwide [26], a staggering number that has likely increased since it was reported over six years ago due to the continuous growth of the industry (see [Production of eggs worldwide 2022 | Statista](#)). Common methods for slaughtering – or ‘culling’ – male chicks include gassing and maceration. With gassing, high concentrations of carbon dioxide are used to kill the chicks, however this can cause hypercapnia (excessive carbon dioxide in the bloodstream) and hypoxia (a deprivation of oxygen) before individuals lose consciousness, meaning that this approach – although generally considered humane – can still inflict pain and suffering [27]. Furthermore, maceration is a way to mechanically decimate the chicks, mostly using a ‘chick shredder’: a high-speed machine with rotating blades [28]. Although generally perceived as cruel by the public, this method – if performed properly – is more fast and painless as compared to gassing [27, 29, 30]. However, the execution of chick shredding can be far from proper, and

hazards with serious, negative welfare consequences are identified in practice, like; blades or rollers that are rotating too slowly, machines that are overloaded (i.e., too many chicks are inserted at once), or rollers that are spaced too widely [31]. These hazards can lead to large numbers of chicks not being killed instantaneously, and thus experiencing prolonged pain, fear, and distress.

Several techniques are under investigation for *in ovo* sexing of chicken eggs, and two major categories of approaches are the spectrometry-based techniques and the molecular techniques. On the one hand, spectrometry-based techniques are relatively fast and do not require DNA extraction, but involve complex data processing steps and can lack accuracy in some cases [32, 33]. On the other hand, molecular approaches, such as genotyping, are highly accurate but require more time and detailed laboratory procedures [32, 34]. The mxKASP set-up, as discussed in **Chapter 3**, would include a DNA extraction step, but eliminates the need for post-PCR analyses (such as checking results on gel, as is the case in standard PCR). Furthermore, KASP is extremely well-suited for high-throughput genotyping, which is essential for a rapid, large-scale usage in farming.

Wildlife Conservation

“In the end, it's not just about the animals, it's about us and our survival as well.” – Steve Irwin

The biodiversity of the world is currently declining rapidly in what is already referred to as the ‘sixth mass extinction’ [35]. For the sake of simplicity and continuity, I will here focus specifically on the decline of amphibian populations. As discussed in **Chapter 1**, amphibians represent the most threatened group of vertebrates and they are, unfortunately, easily affected by anthropogenic impacts on their environments [36-39]. It is estimated that over 70% of the world’s amphibians are currently experiencing population declines, primarily due to human colonization and globalization [36, 40-42]. The six main drivers behind their mass disappearance include; emerging diseases, habitat loss, pollution, climate change and weather extremes, the illegal pet trade, and threats caused by invasive species [37-39, 43-45].

Chapter 4 of this dissertation is primarily relevant to amphibian wildlife conservation, as it highlights a novel and detailed approach that makes it possible to investigate, monitor, and conserve a specific group of amphibians worldwide. Initially, we optimized the target capture protocol (now termed ‘NewtCap’) for studying the balanced lethal system in *Triturus* newts specifically. However the idea to simultaneously test how effective the approach is for studying other research questions in other salamander species appeared a worthwhile pursuit – not primarily for my personal research output,

but more so in terms of future impact. As demonstrated in **Chapter 4**, NewtCap data have proven useful in a broad range of research fields, including conservation genetics.

In discussing the future of studying and protecting amphibians, I want to emphasize their critical role as bioindicators in ecosystems. Firstly, they form an important link in food chains worldwide [46, 47]. Secondly, they are particularly vulnerable to for instance diseases, pollution, and drought due to their permeable skin and limited dispersal capabilities [48-51]. Simply put: the more biodiverse and natural the environment is, the more likely it is to support healthy and diverse amphibian populations – hence the term ‘bioindicator’. As we continue to lose amphibians at an unprecedented rate, it should serve as a warning as a healthy environment is of importance for our own survival as well. Sir David Attenborough mentioned the following on the matter, back in 2008: *“Amphibians are the lifeblood of many environments, playing key roles in the function of ecosystems, and it is both extraordinary and terrifying that in just a few decades the world could lose half of all these species.”* For me personally, it has been a privilege that the *Triturus* project provided me the opportunity to contribute to a study that can be of great importance to the preservation of a wide range of salamanders across the globe.

Science Communication

“If enlightenment was the salad, entanglement is the soup” – Dr. Neri Oxman

The creation of this dissertation has only been possible thanks to the numerous collaborations with; 1) other academic research institutes, 2) specialized (non-academic) research organizations, and 3) citizen scientists and volunteers. In this section, I want to primarily focus on the importance of collaborating and communicating beyond academia (i.e., number 2 and 3). For instance, the *Triturus* embryos that I analyzed in the context of the balanced lethal system – and that thus form the basis for **Chapter 3**, as well as the empirical research in **Chapter 6** – were collected by a hobbyist breeder. Similarly, some of the samples provided to re-build the Salamandridae phylogeny of **Chapter 4** also originated from cooperative hobbyists and pet owners. Additionally, I have, for instance, co-supervised and co-authored student projects that were not part of this dissertation, but that did yield remarkable outcomes for the herpetological sciences [52-66]. Again, these projects were partially made possible through the support of motivated volunteers, as well as practitioners, who assisted with fieldwork and with the publication process. This clearly demonstrates the importance of working together with citizen scientists, hobbyists, and other types of professional experts outside of the academic ‘bubble’.

Non-academic experts and hobbyists often do not only have a deep motivation to help science progress, but they also tend to bring substantial ‘niche knowledge’ that can

significantly enhance the scientific process [67-69]. And people outside of expert circles may be unaware of the studies or research projects conducted by scientific institutes, but they often are eager to learn and become engaged once introduced to a certain subject [70]. Moreover, I strongly believe that the general public can assist scientists in translating their research to broader audiences more effectively (as they themselves are part of that audience). For example, one article I co-authored a few years ago (which is, again, not a part of this dissertation, but which is a part of the overall balanced lethal system project of our research team) got published in *Frontiers for Young Minds*: a scientific journal that is peer-reviewed by highly capable and motivated teenagers [2]. Similarly, **Chapter 2** of this dissertation is based on a popular science article that I wrote to make the balanced lethal system topic accessible not only to the scientific community, but also to experts, volunteers, and hobbyists interested in the aberrant evolution and genetics of crested and marbled newts [71]. While writing it, I specifically consulted non-biologist friends.

Unfortunately, within academia, conducting scientific outreach has traditionally been – and often still is – perceived as a low-status task, usually delegated to or reserved for graduate students and early-career scientists [predominantly women; 72, 73, 74]. Personally, I find engaging with broader audiences not only highly rewarding, but I also consider it an ethical responsibility to educate the public. Whether scientists themselves should be responsible for public engagement remains a topic of debate – and similar debates exist regarding what are the best approaches for doing so [75-77]. Also, similar debates apply to determining how best to direct the future of science. For instance, there is much discussion around 1) how to fairly and effectively evaluate and promote academic performance [78-80], and 2) how to balance academic research and scientific integrity with the increasing interconnectedness between science, industry, and society [81, 82]. And while these issues are beyond the scope of this dissertation, I will offer the following, given my active involvement in public engagement (see Appendices):

Regardless of how the academic landscape evolves; conducting solid research will always be essential. It leads to robust, high-quality science – an intriguing salad. But establishing strong collaborations and communicating science effectively? That is what makes for a big cup of warm soup.

References

1. Wielstra, B., *Balanced lethal systems*. Current Biology, 2020. **30**: p. R742-R743.
2. Meilink, W.R.M., et al., *Balanced Lethal Systems: an evolutionary mystery*. Frontiers for Young Minds, 2021. **9**.
3. Berdan, E.L., et al., *Muller's ratchet and the long-term fate of chromosomal inversions*. bioRxiv, 2019: p. 606012.
4. Berdan, E.L., et al., *Mutation accumulation opposes polymorphism: supergenes and the curious case of balanced lethals*. Philosophical Transactions of the Royal Society B, 2022. **377**(1856): p. 20210199.
5. France, J., et al., *Genomic evidence suggests the balanced lethal system in Triturus newts originated in an instantaneous speciation event*. bioRxiv, 2024; p. 2024.10.29.620207.
6. Sessions, S.K., et al., *Cytology, embryology, and evolution of the developmental arrest syndrome in newts of the genus Triturus (Caudata: Salamandridae)*. The Journal of Experimental Zoology, 1988. **248**: p. 321-334.
7. Giani, A.M., et al., *Long walk to genomics: History and current approaches to genome sequencing and assembly*. Computational and Structural Biotechnology Journal, 2020. **18**: p. 9-19.
8. Friedrich, M., *Evo-Devo gene toolkit update: At least seven Pax transcription factor subfamilies in the last common ancestor of bilaterian animals*. Evolution and Development, 2015. **17**: p. 255-257.
9. Joshi, C.J., et al., *What are housekeeping genes?* PLoS Computational Biology, 2022. **18**(7): p. e1010295.
10. Steinberg, M.H., *Sickle Cell Anemia, the First Molecular Disease: Overview of Molecular Etiology, Pathophysiology, and Therapeutic Approaches*. The Scientific World JOURNAL, 2008. **8**: p. 1295-1324.
11. Herrick, J.B., *Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia*. Archives of internal medicine, 1910. **6**(5): p. 517-521.
12. Pauling, L., et al., *Sickle cell anemia, a molecular disease*. Science, 1949. **110**(2865): p. 543-548.
13. Snow, R.W., et al., *The global distribution of clinical episodes of Plasmodium falciparum malaria*. Nature, 2005. **434**(7030): p. 214-217.
14. Allison, A.C., *The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1954. **48**(4): p. 312-318.
15. *A novel locus of resistance to severe malaria in a region of ancient balancing selection*. Nature, 2015. **526**(7572): p. 253-257.
16. Band, G., et al., *Malaria protection due to sickle haemoglobin depends on parasite genotype*. Nature, 2021. **602**(7895): p. 106-111.
17. Elguero, E., et al., *Malaria continues to select for sickle cell trait in Central Africa*. Proceedings of the National Academy of Sciences, 2015. **112**(22): p. 7051-7054.
18. Habibzadeh, F., *The effect on the equilibrium sickle cell allele frequency of the probable protection conferred by malaria and sickle cell gene against other infectious diseases*. Scientific Reports, 2024. **14**(1).
19. Hochholdinger, F. and J.A. Baldauf, *Heterosis in plants*. Current Biology, 2018. **28**(18): p. R1089-R1092.
20. Liu, J., et al., *Exploring the molecular basis of heterosis for plant breeding*. Journal of Integrative Plant Biology, 2020. **62**(3): p. 287-298.
21. Bruns, H.A., *Southern Corn Leaf Blight: A Story Worth Retelling*. Agronomy Journal, 2017. **109**(4): p. 1218-1224.
22. Sheoran, S., et al., *Recent Advances for Drought Stress Tolerance in Maize (Zea mays L.): Present Status and Future Prospects*. Frontiers in Plant Science, 2022. **13**: p. 872566.
23. Zhu, M., et al., *Genetic dissection of maize disease resistance and its applications in molecular breeding*. Molecular Breeding, 2021. **41**(5): p. 32.
24. Abera, W., et al., *Heterosis and combining ability of elite maize inbred lines under northern corn leaf blight disease prone environments of the mid-altitude tropics*. Euphytica, 2015. **208**(2): p. 391-400.
25. Ahmad, Z., et al., *Studies on screening of maize (Zea mays L.) hybrids under drought stress conditions*. Journal of Advance Botany and Zoology, 2015. **2**: p. 1-5.

26. Krautwald-Junghanns, M.E., et al., *Current approaches to avoid the culling of day-old male chicks in the layer industry, with special reference to spectroscopic methods*. Poultry Science, 2018. **97**(3): p. 749-757.
27. Gurung, S., et al., *Evaluation of Alternative Euthanasia Methods of Neonatal Chickens*. Animals (Basel), 2018. **8**(3).
28. Rutt, R.L. and J. Jakobsen, *The 'brother layer problem': Routine killing, biotechnology and the pursuit of 'ethical sustainability' in industrial poultry*. Environment and Planning E: Nature and Space, 2022. **6**(3): p. 1785-1803.
29. Wang, X., et al., *Evaluation of Euthanasia Methods on Behavioral and Physiological Responses of Newly Hatched Male Layer Chicks*. Animals (Basel), 2021. **11**(6).
30. van Niekerk, T. and J. Workamp, *Scenarios for addressing the dilemma of 'the culling of day-old male chicks of layerbreeds'*. 2022, Wageningen Livestock Research.
31. AHAW, E.P.o.A.H.a.W., et al., *Killing for purposes other than slaughter: poultry*. EFSA Journal, 2019. **17**(11): p. e05850.
32. Jia, N., et al., *A Review of Key Techniques for in Ovo Sexing of Chicken Eggs*. Agriculture, 2023. **13**(3).
33. Galli, R., et al., *In ovo sexing of chicken eggs by fluorescence spectroscopy*. Analytical and Bioanalytical Chemistry, 2017. **409**(5): p. 1185-1194.
34. Corion, M., et al., *Trends in in ovo sexing technologies: insights and interpretation from papers and patents*. Journal of Animal Science and Biotechnology, 2023. **14**(1): p. 102.
35. Ceballos, G., et al., *Accelerated modern human-induced species losses: Entering the sixth mass extinction*. Science advances, 2015. **1**(5): p. e1400253.
36. Stuart, S.N., et al., *Status and trends of amphibian declines and extinctions worldwide*. Science, 2004. **306**: p. 1783-1787.
37. Araujo, A., et al., *Micro(nano)plastics as an emerging risk factor to the health of amphibian: A scientometric and systematic review*. Chemosphere, 2021. **283**: p. 131090.
38. Hof, C., et al., *Additive threats from pathogens, climate and land-use change for global amphibian diversity*. Nature, 2011. **480**(7378): p. 516-9.
39. Ford, J., et al., *Adrift on a Sea of Troubles: Can Amphibians Survive in a Human-Dominated World?1*. Herpetologica, 2020. **76**(2).
40. Hayes, T.B., et al., *The cause of global amphibian declines: a developmental endocrinologist's perspective*. Journal of Experimental Biology, 2010. **213**: p. 921-933.
41. Sparreboom, M., *Salamanders of the Old World: the salamanders of Europe, Asia and northern Africa*. 2014: p. 431p.
42. Pabijan, M., et al., *Evolutionary principles guiding amphibian conservation*. Evolutionary Applications, 2020. **00**: p. 1-22.
43. Hughes, A.C., B.M. Marshall, and C.T. Strine, *Gaps in global wildlife trade monitoring leave amphibians vulnerable*. eLife, 2021. **10**.
44. Stringham, O.C., J.L. Lockwood, and C. Bellard, *Pet problems: Biological and economic factors that influence the release of alien reptiles and amphibians by pet owners*. Journal of Applied Ecology, 2018. **55**(6): p. 2632-2640.
45. Lucas, P.M., et al., *Using comparative extinction risk analysis to prioritize the IUCN Red List reassessments of amphibians*. Conservation Biology, 2024: p. e14316.
46. West, J., *Importance of amphibians: A synthesis of their environmental functions, benefits to humans, and need for conservation*. 2018.
47. Gobel, N., et al., *Invariant and vulnerable food web components after bullfrog invasion*. Biological Invasions, 2022. **25**(3): p. 901-916.
48. Valencia-Aguilar, A., A.M. Cortés-Gómez, and C.A. Ruiz-Agudelo, *Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems*. International Journal of Biodiversity Science, Ecosystem Services & Management, 2013. **9**(3): p. 257-272.
49. Hocking, D.J. and K.J. Babbitt, *Amphibian contributions to ecosystem services*. Herpetological conservation and biology, 2014.
50. Van der Goot, A., M. De Visser, and A.F. Hiemastra, *Smooth newts *Lissotriton vulgaris* observed hibernating in a waterfowl nest*. Herpetological Bulletin, 2022. **162**: p. 41-42.
51. Saber, S., W. Tito, and R. Said, *Amphibians as Bioindicators of the Health of Some Wetlands in Ethiopia*. The Egyptian Journal of Hospital Medicine, 2017. **66**: p. 66-73.

52. Kalaentzis, K., et al., *Hybrid zone analysis confirms cryptic species of banded newt and does not support competitive displacement since secondary contact*. Ecology and Evolution, 2023. **13**(9): p. e10442.
53. Kazilas, C., et al., *Spatial genetic structure in European marbled newts revealed with target enrichment by sequence capture*. Molecular Phylogenetics and Evolution, 2024. **194**: p. 108043.
54. Struijk, R.P., et al., *Knoflookpad in Callantsoog: Verspreiding, voortplanting en geografische herkomst*. RAVON, 2024. **26**(1): p. 2-5.
55. Kuijt, M., et al., *The introduction of three cryptic tree frog species in the Dutch coastal dunes challenges conservation paradigms*. Amphibia-Reptilia, 2022. **44**(1): p. 1-10.
56. Koster, S., et al., *The conservation paradox of an introduced population of a threatened species: spadefoot toads in the coastal dunes of the Netherlands*. Amphibia-Reptilia, 2022. **44**(1): p. 11-18.
57. de Brouwer, J., et al., *An isolated crested newt population in Dutch coastal dunes: distribution relict or introduction?* Amphibia-Reptilia, 2022. **44**(1): p. 19-26.
58. Vliegthart, C., et al., *The limits of mtDNA analysis for determining the provenance of invasive species: a midwife toad example*. Amphibia-Reptilia, 2022. **44**(1): p. 27-33.
59. De Visser, M., et al., *Exotische amfibieën in de duinen ontmaskerd met mtDNA barcoding*. Holland's Duinen, 2023. **82**(1): p. 25-29.
60. Fahrbach, M., M. de Visser, and B. Wielstra, *The hybrid zone between the Italian and Northern Crested Newts (*Triturus carnifex* and *T. cristatus*) reaches Germany*. Salamandra, 2021. **57**(3).
61. Struijk, R.P., et al., *'Rise and fall' van een populatie westelijke smaragdhagedissen in Scheveningen*. RAVON, 2022. **24**(2): p. 24-27.
62. Struijk, R.P., M. de Visser, and B. Wielstra, *Exotische smaragdhagedissen in de duinen bij Scheveningen*. Kijk op Exoten, 2021. **9**(3): p. 12-13.
63. Wielstra, B., et al., *Herkomst van exotische Alpenwatersalamanders blootgelegd*. Schubben & Slijm, 2023. **54**: p. 8-9.
64. Wielstra, B., et al., *MtDNA barcoding van exotische amfibieën in de duinen*. RAVON, 2023. **25**(2): p. 26-29.
65. Mars, J., et al., *Phylogenomics yields new systematic and taxonomical insights for *Lissotriton* newts, a lineage with a strong legacy of introgressive hybridization*. SSRN Preprints, 2024.
66. Robbemont, J., et al., *An extended mtDNA phylogeography for the alpine newt illuminates the provenance of introduced populations*. Amphibia-Reptilia, 2023. **44**(3): p. 347-361.
67. Jones, M.G., et al., *Citizen scientists and non-citizen scientist hobbyists: Motivation, benefits, and influences*. International Journal of Science Education, Part B, 2018. **8**(4): p. 287-306.
68. Kobori, H., et al., *Citizen science: a new approach to advance ecology, education, and conservation*. Ecological Research, 2015. **31**(1): p. 1-19.
69. Bonney, R., et al., *Can citizen science enhance public understanding of science?* Public Understanding of Science, 2016. **25**(1): p. 2-16.
70. Jensen, E. and N. Buckley, *Why people attend science festivals: Interests, motivations and self-reported benefits of public engagement with research*. Public Underst Sci, 2014. **23**(5): p. 557-73.
71. De Visser, M., et al., *Een evolutionair raadsel: het dodelijke chromosoom 1 syndroom in *Triturus*-salamanders*. RAVON, 2021. **23**(1): p. 9-12.
72. Woitowich, N.C., et al., *Assessing Motivations and Barriers to Science Outreach within Academia: A Mixed-Methods Survey*. bioRxiv, 2021: p. 2021.10.28.466319.
73. Johnson, D.R., E.H. Ecklund, and A.E. Lincoln, *Narratives of Science Outreach in Elite Contexts of Academic Science*. Science Communication, 2013. **36**(1): p. 81-105.
74. Bankston, A. and G.S. McDowell, *Changing the Culture of Science Communication Training for Junior Scientists*. Journal of Microbiology & Biology Education, 2018. **19**(1).
75. Peacock, K.A., *The Duty to Communicate*. BioScience, 2020. **70**(8): p. 719-721.
76. Gardner, C.J., et al., *From Publications to Public Actions: The Role of Universities in Facilitating Academic Advocacy and Activism in the Climate and Ecological Emergency*. Frontiers in Sustainability, 2021. **2**.
77. Llorente, C., et al., *Scientists' opinions and attitudes towards citizens' understanding of science and their role in public engagement activities*. PLoS ONE, 2019. **14**(11): p. e0224262.
78. Edwards, M.A. and S. Roy, *Academic Research in the 21st Century: Maintaining Scientific Integrity in a Climate of Perverse Incentives and Hypercompetition*. Environmental Engineering Science, 2017. **34**(1): p. 51-61.

79. Kassab, O., *Does public outreach impede research performance? Exploring the 'researcher's dilemma' in a sustainability research center.* Science and Public Policy, 2019. **46**(5): p. 710-720.
80. Herbaut, E. and K. Geven, *What works to reduce inequalities in higher education? A systematic review of the (quasi-)experimental literature on outreach and financial aid.* Research in Social Stratification and Mobility, 2020. **65**.
81. Mecca, J.T., et al., *Researcher Perspectives on Conflicts of Interest: A Qualitative Analysis of Views from Academia.* Science & Engineering Ethics, 2015. **21**(4): p. 843-55.
82. Johns, M.M., M. Barnes, and P.S. Florencio, *Restoring balance to industry-academia relationships in an era of institutional financial conflicts of interest: promoting research while maintaining trust.* Journal of the American Medical Association, 2003. **289**(6): p. 741-746.