Light at the end of the tunnel: Insight into the evolution of bioluminescence

Bachelor Thesis

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Date: 30-6-2023

Summary

This literature research aims to give insight into the evolutionary history of bioluminescence. Bioluminescence can be defined as the emission of light by living organisms. The following research question: “What are the modes of evolution when looking at bioluminescence systems based on luciferin and luciferases or photoproteins that have evolved over time?” was created in an attempt to shed light on the evolutionary mysteries of the emission of light. Firstly, it was expected that that convergent evolution could explain the evolutionary history between the different luciferins responsible for bioluminescence. Furthermore, it was hypothesized that when looking at the separate light-emitting systems categorized per luciferin, convergent evolution will prove insufficient to explain their evolutionary history. Concludingly, it can be said that convergent evolution prevails when the different luciferins are evaluated between each other. When looking at the different luciferins individually it can be said that tetrapyrrole and cypridina have likely evolved convergently, while parallel evolution is the main mode of evolution in coelenterazine and D-luciferin. Lastly, it was determined that light emission in bacteria and fungi could in both cases are an example of divergent evolution. Overall, this thesis aims to provide insight into the existing knowledge of the evolutionary history of bioluminescence and reveal underexposed aspects that require more extensive research.

Contents

Introduction

The emission of light by living organisms was already described all the way back in 350BC by Aristotle in his De Anima and described it as a type of light, unlike the flame of a candle due to the absence of heat, visible in dead fish and flesh (Lee, 2008). Throughout the centuries it found its way into for example the stories of seafarers and the myths of the Siberians and Scandinavians (Harvey, 1957).

However, in this day and age, this phenomenon is more commonly known as bioluminescence. The ability to emit light has emerged on numerous occasions in evolutionary time and is considered widespread across the tree of life (Haddock et al., 2010). So far, it has been described in at least 700 genera belonging to many different lineages, such as bacteria, arthropods and even certain species of fish (Delroisse et al., 2021).

The origin of bioluminescence dates back to the early days of life on Earth. It is expected that this trait emerged around 2400 million years ago, the time in which the concentration of oxygen on Earth started to take on significant levels (Wilson, 2013). This coincides with the current hypothesis for the origin of bioluminescence which is that it was used as a defensive mechanism against oxygen. This idea is founded upon the realization that oxygen and its derivatives would be toxic to the strictly anaerobic cells at the time (Wilson, 2013). As all known bioluminescence systems use some variety of oxygen, but mainly O_2, it could have played a role in neutralizing this oxygen rendering the emission of light as merely a side effect of this defensive mechanism (Rees et al., 1998).

While, bioluminescence looks very impressive, often its chemical foundation is surprisingly straightforward. The first method involves two components, so-called luciferins and luciferases that are responsible for this light emission by many organisms. Luciferins are the small molecules that upon oxidation have the ability to emit light. This process is then catalyzed by certain enzymes, called luciferases (Fleiss & Sarkisyan, 2019).

On the other hand, there are also organisms that make use of so-called photoproteins. These can for example be found in several shark families such as *Etmopteridae* and *Dalatiidae*. Photoproteins differ from the aforementioned light emission systems in that a complex molecule is formed that consists of both a preoxidized luciferin as well as a luciferase (Duchatelet et al., 2021). Because the luciferin is already oxidized, light emission using this photoproteins is independent of the presence of molecular oxygen at the moment light is actually emitted (Eremeeva & Vysotski, 2018).

Due to the abundance of bioluminescence in the tree of life, it is unsurprising that there are many different types of these luciferin-luciferase systems to be distinguished. In total it is expected in nature that there are at least 40 different luminescent systems. However, of only nine of these systems the structures of the luciferin molecules are known. This number is further reduced when it is taken into account that only of seven of these systems, at least one luciferase gene has been identified (Fleiss & Sarkisyan, 2019). It will then also these seven bioluminescent systems that will form the basis of the different categories to be evaluated for their long journey through evolutionary time.

However, apart from bioluminescence being a fascinating phenomenon, it is also very useful in for example biotechnology. It is used in so-called bioluminescence-based probes that allow for both the perceiving of biological processes as well as altering their functionality (Jiang et al., 2023). However, this is only the tip of the iceberg as this feature also found its way into a wide range of other fields, from guaranteeing in fish and milk industries to gaining insight into the levels of pollution in ecosystems (Syed & Anderson, 2021).

While our understanding of bioluminescence increases day by day when it comes to discovering new luciferin molecules and luciferases involved as well as their potential usage in biotechnology, a significant part remains unknown about the evolutionary history of bioluminescence. In an attempt to shed light on the mysteries regarding the evolution of bioluminescence the following research question is formulated: “What are the modes of evolution when looking at bioluminescence systems based on luciferin and luciferases or photoproteins that have evolved over time?”. The first corresponding hypothesis is that convergent evolution is able to explain the evolutionary history between the different luciferins responsible for bioluminescence because bioluminescence is a phenomenon that has arisen independently in a plethora of different genera from all over the tree of life. The second hypothesis is that when looking at the separate light-emitting systems categorized per luciferin, convergent evolution will prove insufficient to explain their evolutionary history.

There are three main types of evolution: convergent, divergent and parallel evolution. To allow for an objective evaluation, it might be important to formerly define these modes of evolution. Divergent evolution can be defined as “Divergent evolution represents the evolutionary pattern in which species sharing a common ancestry become more distinct due to differential selection pressure which gradually leads to speciation over an evolutionary time period” (Gautam, 2020). For this thesis the definition of convergent evolution is “The independent appearance in different lineages of similar derived characters” (Herron & Freeman, 2014).

Along similar lines, there is parallel evolution which is a bit more ambiguous to define and is sometimes used interchangeably in literature or seen as subset of convergent evolution. However, there is one distinction that allows it to have its definition, which is that in parallel evolution contrary to convergent evolution the species in question are descendants of the same ancestor, which is not the case in convergent evolution. Therefore, parallel evolution can be defined as: “the acquisition of the same trait in species descending from the same ancestor but connected by non-continuous lineages” (Elias & Tawfik, 2012).

For this thesis, the most relevant current literature available regarding the evolution of bioluminescence is tested against the aforementioned definitions of the types of evolution. The following sections are divided according to the different luciferins to keep a clear overview of the material presented.

Results

Coelerentazine luciferin

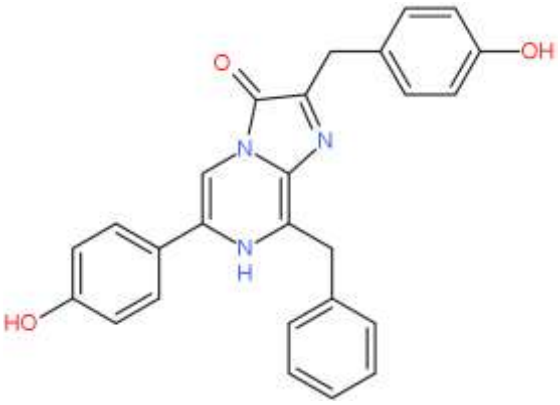

Figure 1: The molecule structure of coelenterazine luciferin

The luciferin coelenterazine (figure 1) can be considered widespread as it is found in many marine organisms capable of bioluminescence (Jiang et al., 2016). Actually, marine ecosystems harbor the highest diversity of bioluminescent species of any habitat (Fleiss & Sarkisyan, 2019). Therefore, it is unsurprising that this luciferin has been found in species belonging to 9 different phyla: *Arthropoda*, *Chaetognatha*, *Chordata*, *Cnidaria*, *Ctenophora*, *Echinodermata*, *Mollusca*, *Protozoa* and finally *Porifera* (Vassel et al., 2012 ; Martini et al., 2020). Most of the organisms that belong to these phyla do not have the ability to synthesize coelenterazine themselves and are therefore dependent on obtaining it from their diet (Eremeeva et al., 2020). Nonetheless, Oba et al. (2009) described the ability of the copepod *Metridia* in regard to the biosynthesis of coelenterazine, while Thomson et al. (1995) found strong indications that a certain deep sea shrimp by the name of *Systellaspis debilis*, could do the same.

Interestingly, coelenterazine functions as a substrate for both luciferases as well as photoproteins. Mainly in cnidarians and ctenophores, coelenterazine is present in preoxidized form in the aforementioned photoproteins that are regulated by Ca^{2+} (Eremeeva et al., 2020). These Ca-ions were found to have a great beneficial impact on the light-emitting reaction generated by photoprotein. While even in absence of calcium, photoproteins produce a faint glow, the addition of Ca^{2+} escalates the emission of light up to a one million fold (Eremeeva et al., 2020).

On the other hand, for example copepods, decapods and ostracods, make use of the “traditional” luciferin-luciferase bioluminescent systems that do not make use of other cofactors (Inouye et al., 2013). Merely the presence of O_2 is enough for the emission of light in these instances (Fleiss & Sarkisyan, 2019).

Coelenterazine is also used quite extensively for biotechnological purposes. This is partly due to the fact that Inoue et al. (1976) already managed to isolate this luciferin and unravel its molecular structure. This means that it was one of the first luciferins to be discovered and is therefore logically one of the most often employed bioluminescent systems (Syed & Anderson, 2021). Secondly, this luciferin has a low toxicity, making it a powerful tool in biomedical research (Krasitskaya et al., 2020). Lastly, it has a high reaction sensitivity, which allows it to be effective even at low concentrations (Syed & Anderson, 2021).

However, what about the evolutionary origins of coelenterazine-based bioluminescent systems? Firstly, it has to be stated that this is a complicated group because it spans over 9 different phyla and as mentioned earlier by no means all organisms belonging to these phyla actually produce coelenterazine themselves but instead acquire it through their food intake. However, there are two main hypotheses when it comes to the evolution of bioluminescence that involves coelenterazine, which is convergent evolution and parallel evolution.

It seems that the general consensus is that between phyla that use coelenterazine, convergent evolution prevails due to the many different independent origins of bioluminescence (Lau & Oakley, 2020). This idea is further supported by the identification of a putative chordate luciferase that points in the direction of convergent evolution between Cnidaria, Echinodermata, and Chordata (Tessler et al., 2020). A few years prior Echinodermata was already proposed to have a convergent connection to Cnidaria because of the discovery of homologous luciferases between these phyla (Delroisse et al., 2017). Normally, homologous genes are an indication of a common ancestor but according to Delroisse et al. (2017) this puzzling homology can be explained by that the different luciferases were co-opted independently from very similar genes, therefore still carrying their similarity to this day. On the other hand, there are also voices that claim a potential for parallel evolution of photoproteins between *Cnidaria* and *Ctenophora* due to the discovery of conserved homologous genes between these phyla (Delroisse et al., 2021).

The trend of independent origins of bioluminescence seems to continue when inspecting the evolutionary history within phyla. For example, in cnidarians it was found that bioluminescence emerged at least 6 times in this phylum (Bessho-Uehara et al., 2020). However due to the definition used in this thesis, this would be a case of parallel evolution due to their distant relatedness.

Furthermore, there are also several phyla of which less information is currently known. For example, in the *Porifera* where only recently a deep-sea sponge was identified that was capable of bioluminescence (Schultz, 2021). However, it should be noted that its credibility is not optimal as this source is still under embargo and not published anywhere. The same holds true for the *Echinodermata,* where in crinoids it was determined that more research would be needed to provide a definitive answer regarding their evolutionary history (Mallefet et al., 2023). Another example is the phylum *Protozoa*, where there seems to be a knowledge gap in regards to their type of evolution.

Meanwhile, in *Chordata* bioluminescence is observed from sharks to tunicates (Duchatelet et al., 2021; Tessler et al., 2020). In this phylum both photoprotein systems as well as traditional luciferin-luciferase systems can be found (Duchatelet et al., 2021). Therefore, it is safe to say that there are multiple different origins of bioluminescence within this group, making it an example of parallel evolution.

According to Lindgren et al. (2012), strong evidence was found that in *Cephalodpods*, a member of the phylum *Mollusca*, convergent evolution is the basis for light emission. For this thesis, convergent evolution will once again have to be rewritten to parallel evolution. Along similar lines, there is evidence that between two distantly related species belonging to the phylum *Chaetognatha* have evolved convergently concerning bioluminescence (Thuesen et al., 2010), which for this thesis is of course known as parallel evolution.

Lastly, in *Ctenophores* there are indications that the photoproteins in this phylum emerged from a common *Metozoan* ancestor, making it likely that at least the photoproteins in this phylum

originated from divergent evolution (Delroisse et al., 2021). However, it remains unclear how the traditional luciferin-luciferase systems fits into this phylum.

Cypridina luciferin

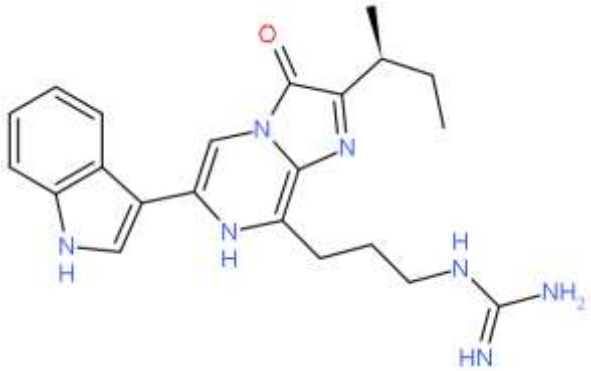

Figure 2: The molecule structure of cypridina luciferin.

Cypridina luciferin, also referred to as Vargula luciferin, Vargulin and cypridinid luciferin can, just like coelenterazine be found in marine ecosystems (figure 2). However, contrary to coelenterazine, this luciferin becomes unstable when it comes into contact with atmospheric oxygen which made its initial separation not an easy task (Jiang et al., 2023). Nevertheless, Shimomura et al. (1957) were the first to succeed in isolating Cypridina luciferin from *Cypridina Hilgendorfii*. In the ostracod family *Cypridina*, consisting of more than 300 members, approximately half are able to emit light (Morin, 2019). While, it is to date still unknown whether all of these species make use of Cypridina for their bioluminescence, it should be noted that every organism that has been investigated does indeed use this luciferin (Delroisse et al., 2021).

Furthermore, this luciferin has also been determined in three different lineages of fish: *Apogonidae*, *Batrachoididae* and *Pempheridae* (Bessho-Uehara et al., 2020). A member of *Batrachoididae*, the midshipman fish *Porichthys*, likely obtains their luciferin from their diet of ostracods (Mensinger & Case, 1991), while Parapriacanthus *ransonneti*, belonging to the lineage Pempheridae, takes it even one step further as it probably also hijacks the corresponding luciferase from their prey (Bessho-Uehara et al., 2020). Lastly, the bioluminescent reaction with Cypridina is quite basic as it is not dependent on any other cofactors and thus simply requires the combination of luciferin, luciferase and oxygen (Kaskova et al., 2016).

It proves quite challenging to assess the evolutionary history of the group organisms that make use of cypridina as their luciferin. This is partly because, like the wide variety of names for this luciferin suggests, there is some difficulties regarding terminology. So much so, that even a paper was written with the aim of deciphering the most inclusive way of how one should refer to this luciferin, which was determined to be cyprinid luciferin (Morin, 2011). The reason for this situation is that the genus Cypridina has a long taxonomic history where due to the discovery of many new species the phylogentic overview has been lost (Morin, 2011). Even today, the genus *Vargula* has been described as a “catch-all” genus where many species are simply filed under while they actually should be moved to other genera that are for now still undescribed (Morin, 2019).

Another complicating factor is that convergent evolution has been the supposed hypothesis for several coastal species of fish that use cypridinid luciferin, such as the aforementioned lineages of *Apogonidae*, *Batrachoididae* and *Pempheridae* because it was deemed likely that the luciferases would be analogous between the ostracods and fish lineages (Waldenmaier et al., 2012). However, with the recent discovery of “kleptoproteins” (Bessho-Uehara et al., 2020), this theory of convergent evolution, or divergent and parallel evolution for that matter is called into question.

Nontheless, when merely zooming in on the ostracods that use cypridina as their luciferin it seems that this bioluminescent system evolved only once (Morin, 2011), leading to the proposition that divergent evolution underpins the emergence of bioluminescence in this group of ostracods.

D-luciferin

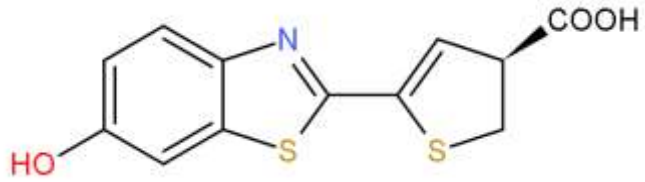

Figure 3: The molecule structure of D-luciferin

Bioluminescent systems based around D-luciferin are among the most well-studied groups of bioluminescence (figure 3). It was also the first luciferin that was discovered and purified all the way back in 1949 (Strehler & McElroy, 1949). This luciferin is used by approximately 40 different species that belong to lineages such as fireflies, click beetles and railroad worms (Syed & Anderson, 2021). Furthermore, there are also several starworms that have engage in bioluminescence using the same luciferin (Fallon et al., 2018).

Logically, it used very extensively in all kinds of applications ranging from measuring bacterial contamination of drinking water (Frundzhyan & Ugarova, 2007), to understanding cancer metabolism (Patergnani et al., 2014). It especially thrives when it is used for visualizing processes where blood is involved as it has a long emission wavelength that is not as effectively absorbed by hemoglobin and surrounding tissues (Syed & Anderson, 2021).

It has very similar properties to coelenterazine, which as was mentioned before is also often used in the biotechnological field, as the reactions involving D-luciferin tend to be quite stable (Fleiss & Sarkisyan, 2019). It is also a bit less toxic and better soluble in water than coelenterazine(Syed & Anderson, 2021). Moreover, it does have an additional benefit as it can cross the blood-brain barrier unlike coelenterazine (Kaskova et al., 2016).

For their bioluminescence, both click beetles and fireflies use the “standard” system of oxidizing D-luciferin with the help of a luciferase. Like many other bioluminescent systems O_2 is also needed but interestingly, Mg-ATP is an additional cofactor that is required for this system to emit light (Jiang et al., 2023).

The aforementioned lineages of bioluminescent species all belong to the superfamily *Elateroidea* (Arnoldi et al., 2010; Fallon et al., 2018). It was found that it is likely that click beetles and fireflies have developed their bioluminescent systems independently (Fallon et al., 2018). This would be a clear case of parallel evolution as both luminescent systems emerged

independently while the lineages are distantly related. It becomes more complicated when evaluating the connection between the starworms (*Rhagophthalminae*) and railroad worms (*Phengodini*), where it seems that the luciferases originating from the lateral lanterns of *Mastinocerini* (which is a railroad worm subfamily) are homologous between the groups while the luciferases present in the head lanterns appear paralogous (Arnoldi et al., 2010). However, the evolutionary history of *Phengodini* for now remains unknown (Arnoldi et al., 2010).

Furthermore, it is important to note that D-luciferin is also used by a few *Diptera*. For example, *Arachnocampa richardsae*, also known as the Australian glow-worm, uses D-luciferin as well as a luciferase homologous to *Elateroidea* , given that it is an acyl-CoA synthetase, to emit light (Trowell et al., 2016). According to Trowell et al. (2016), their obtained sequence data suggest that both *Diptera* and *Coleoptera* used the same ancestral gene family, the acyl-CoA synthetases, independently to form the building blocks of the currently used luciferases by both phyla, which would suggest a convergent origin.

Tetrapyrrole-based luciferins

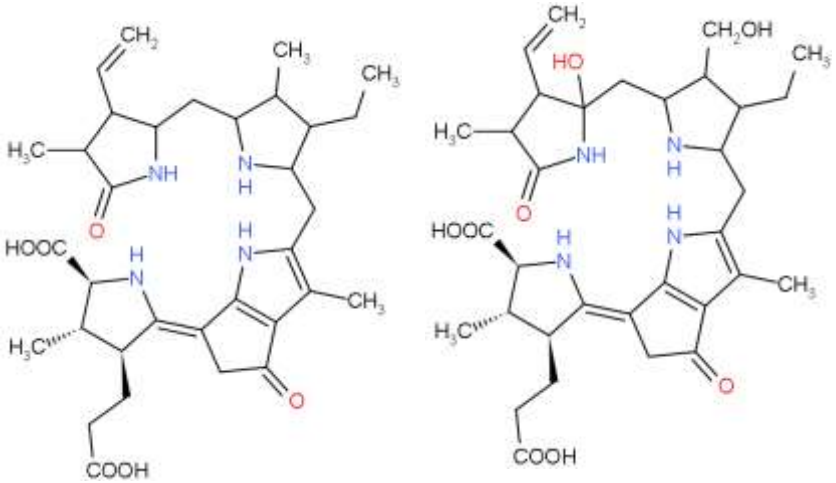

Figure 4: molecule structures of tetrapyrrole-based luciferins. Left is Dinoflagellate luciferin and right is Euphausiid luciferin.

This group features two different luciferins that have a very similar molecule structure (figure 4). These so-called tetrapyrrole luciferins can be found in Dinoflagellates and Euphausiids, also known as krill (Tsarkova, 2021). The fact that these molecules share very high similarity is quite unusual in the world of bioluminescence as most luciferins are unique (Lau & Oakley, 2020). An often mentioned hypothesis is that krill takes up the Dinoflagellate luciferin via their diet (Tsarkova, 2021; Lau & Oakley, 2020; Haddock et al., 2010; Dunlap et al., 1980). However, as no verification has been reported regarding the ingestion of Dinoflagellate luciferin by Euphausiids, this theory to date remains unsupported on account of a lack of evidence (Ramesh & Bessho-Uehara, 2021).

Secondly, on a structural level, both of these luciferins appear quite similar to chlorophyll, leading to the idea that Dinoflagellate luciferin might be derived from chlorophyll (Lau & Oakley, 2020). However, this hypothesis can also be put in question as there are indications that tetrapyrrole luciferin in Dinoflagellates is actually synthesized from an earlier intermediate of which chlorophyll would be derived instead (Janouškovec et al., 2016).

Currently, luciferases from either Dinoflagellates or Euphausiids are only sporadically used in research as no synthetic luciferin analogs are available. It also does not help that tetrapyrrole is an unstable molecule that tends to become inactivated in circumstances with low pH (Dunlap et al., 1980).

The bioluminescence system used by Dinoflagellates is quite remarkable. The production of light happens in so-called scintillons, specialized organelles that contain both the luciferase and luciferin as well as a luciferin-binding protein in most instances (Fajardo et al., 2020). These luciferin-binding proteins are generally involved in protecting against autoxidation and bind the luciferin at physiological pH , a welcome sight for a luciferin not fond of acidic conditions (Fajardo et al., 2020).

On the other hand, *Meganyctiphanes norvegica*, representing the Euphausiids, has 10 separate photophores that emit light (Krönström et al., 2007). While a significant amount remains unknown about the control of light emission in this family, it was found that nitric oxide, a neuromodulator, might fulfill a regulating role in the amount of light produced (Krönström et al., 2007).

Evaluating this group of tetrapyrrole-based luciferins is quite difficult because as mentioned before *Euphasiids* and *Dinoflagellates* make use of albeit very similar but nevertheless different luciferins. Because this category can be seen as slightly unorthodox, it might be beneficial to first scrutinize *Euphasiids* and *Dinoflagellates* separately.

When investigating the evolution of dinoflagellates, some evidence is available that point in the direction of divergent evolution. For example, it was found that, after comparing luciferase genes of seven different *Dinoflagellate* species, these luciferases possibly have a common origin (Liu et al., 2004). Interestingly, there are indications that in the tetrapyrrole luciferin used by dinoflagellates might have its evolutionary origin in their plastids. This would suggest that at least a subset of this phylum actually biosynthesizes this luciferin themselves (Fajardo et al., 2020). Furthermore, it is hypothesized that in all core dinoflagellates a single tetrapyrrole pathway exists which can be traced back to a plastid origin (Janouškovec et al., 2016). Within krill, the evolutionary origin of bioluminescence appears understudied. Still, it was mentioned that for at least the pelagic phyla, such as *Meganyctiphanes norvegica*, light emission had its foundation in convergent evolution (McFall-Ngai, 1990). However, this statement appears unfounded by evidence.

However, when the different luciferases of both *Euphasiids* and *Dinoflagellates* are considered, it becomes clear that these are analogous between these two groups, making it a clear example of convergent evolution (Waldenmaier et al., 2012).

Bacterial luciferin

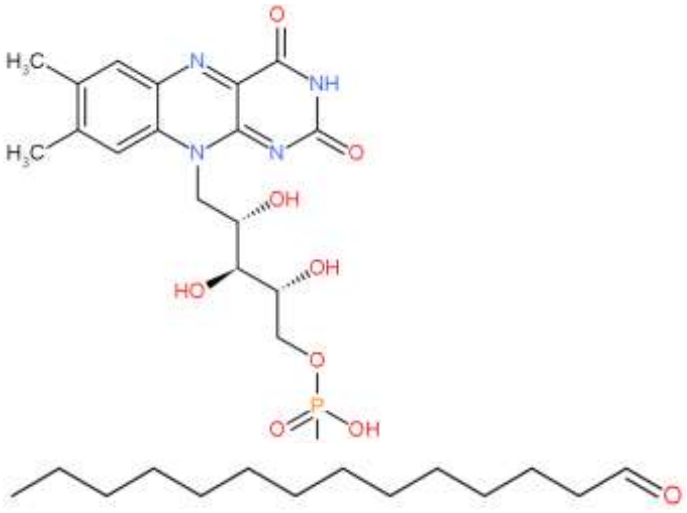

Figure 5: The molecule structure of the two bacterial luciferins. Above is flavin mononuceleotide and under is a long-chain aldehyde.

There are thirty or more bacterial species of which strains exist that have the ability to emit light. All these bacteria are gram-negative and belong to one of the following three families: *Enterobacteriaceae*, *Shewanellaceae* and *Vibrionaceae* (Dunlap, 2014).

While bacteria make use of the traditional luciferin-luciferase system in combination with O_2, there is still one important distinction that makes it unique. In a way, it can be said that bacteria use two luciferins (figure 5), a long-chain aliphatic aldehyde as well as a reduced flavin mononucleotide (Liu, 2022). The flavin mononucleotide is sometimes depicted as the main luciferin as it is “a bit more directly involved”, such as by (Liu, 2022). On the other hand, (Fleiss & Sarkisyan, 2019) favored the long-chain aliphatic aldehyde to be the most substantial luciferin.

However, upon closer inspection it becomes clear that the reduced flavin mononucleotide is the one that that actually undergoes the bioluminescent reaction but only after the long-chain aldehyde has delivered the energy necessary for the flavin molecule elevate to an excited state (Brodl et al., 2018). Even though it is difficult to determine which molecule is more important, it should be said that without the presence of both molecules, a successful emission of light cannot be achieved.

Contrary to many other organisms, bacteria are able to emit light continuously. This is because they are single-celled creatures that already have everything they need at their disposal to produce light (Liu, 2022). As both the long-chain aldehyde is used during the reaction, this also means that the bacterial cell will have to synthesize this molecule at a rapid rate to keep the reaction going (Widder, 2010). However, in regards of the flavin mononucleotide, this luciferin is brought back to its oxidized ground state (Brodl et al., 2018). This means that it will have to converted back to a reduced state quickly enough for flavin to participate in the reaction once again.

Similar to fungal bioluminescence, two different hypotheses can be formulated in an attempt to explain the emergence of light emission in bacteria. The first theory is once again based on convergent evolution, while the latter falls under divergent evolution (Brodl et al., 2018). The comparisons do not end there as the hypothesis in favor of convergent evolution seems

underexposed in the current literature. However, the idea that bacterial bioluminescence evolved from a single origin is declared in multiple papers. For example, Dunlap (2009) already found a widespread homology of the bacterial lux genes, which are necessary to get the bioluminescent system in bacteria up and running, which likely means that bioluminescence in bacteria emerged once in evolutionary time. A supposition that seems to stand the test of time as in a paper from 2014 by the same author, the same conclusion once again came to light (Dunlap, 2014).

Fungal luciferin

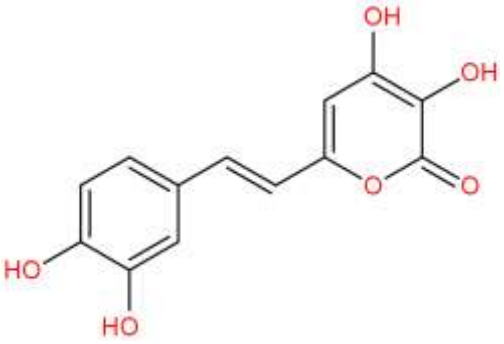

Figure 6: The molecule structure of fungal luciferin.

Fungal luciferin is one of the more recently discovered luciferins as its molecule structure has only been known since 2015 (Purtov et al., 2015). There are roughly 100 species of fungi that are able of bioluminescence (Liu, 2022). At least 71 of these species live in terrestrial ecosystems and can be found all over the world, from Asia to Europe and from Africa to North and South America (Stevani et al., 2013). Their lifestyle is predominantly saprotrophic, meaning that they feed on decaying organic matter, although some fungi are plant pathogenic (Desjardin et al., 2008). All these light-emitting fungi belong to the Agaricales, the order of mushroom-forming fungi, and more specifically can be filed under four different lineages: *Armillaria*, *Lucentipes*, *Mycenoid* and *Omphalotus* (Liu, 2022). Bioluminescent fungi, similar to most other fungi, tend to prefer tropical and temperate environments with an increased humidity and temperature (Stevani et al., 2013).

Under the cap, bioluminescent fungi make use of the traditional method of luciferin, luciferase and oxygen, just like many other organisms (Stevani et al., 2013). For fungi, the process of light emission transpires in three steps. First, hispidin which is the luciferin precursor is oxidized to 3-hydroxyhispidin, also known as luciferin. In step two, luciferin is once again oxidized with help of a luciferase enzyme to an excited oxyluciferin. The last step is where the emission of light takes place, after which oxyluciferin returns to its ground state (García-Iriepa et al., 2020). Just like bacteria, fungi are also able to produce light at a constant rate (Liu, 2022). This is likely due to the ability of fungi to engage in slow recycling biosynthesis of the used luciferin (Oba et al., 2017).

When it comes to the evolution of bioluminescence in fungi, there are two main hypotheses that can be formulated. Firstly, there was a common ancestor that possessed bioluminescent abilities which would be a case of divergent evolution (Ke & Tsai, 2022). Secondly, bioluminescence evolved independently on numerous occasions in evolutionary time, which is also known as convergent evolution (Ke & Tsai, 2022). While for the theory of convergent

evolution it is difficult to acquire evidence supporting this standpoint, the more can be found that bolsters the hypothesis of divergent evolution.

Oliveira et al. (2013) already mentioned, because fungal bioluminescence can be found in many different lineages, that it was likely that this trait evolved early on in evolutionary history and presumably was lost in time on numerous occasions but also reappeared in multiple instances. This idea was further reinforced when it was found, by employing bioinformatic analysis of genomes and transcriptomes, that it was indeed likely that bioluminescence in fungi only emerged once (Kotlobay et al., 2018). A similar study by Ke et al. (2020) also set out to explore the evolution of fungal bioluminescence which resulted in a very similar conclusion that a common ancestor of the *mycenoid* and the *marasmioid* clade was the first fungus able to emit light.

Interestingly, the hypothesis of divergent evolution was preferred even though this did not yield the highest parsimony. Parsimony is a criterion that allows to differentiate between alternative patterns based on minimizing the amount of changes in a model (Herron & Freeman, 2014). This was explained by the fact that all fungus luciferases are homologous and therefore somehow connected through evolutionary time (Ke et al., 2020). Furthermore, it is important to note that the phylogenetic trees produced in the two aforementioned studies do not fully match (Ke & Tsai, 2022).

Discussion

Beforehand, it was expected that convergent evolution could explain the evolutionary history between the different luciferins responsible for bioluminescence. Furthermore, it was hypothesized that when looking at the separate light-emitting systems categorized per luciferin, convergent evolution will prove insufficient to explain their evolutionary history.

When simply comparing between the different luciferins themselves, convergent evolution is most likely the mode of evolution. This is because there are many different luciferins on Earth that all have their own sophisticated reactions from simple systems where a single luciferase is involved to complex photoproteins that are built into elaborate light organs. Also, bioluminescence is present in many different lineages which include, among others beetles, bacteria, fungi and even sharks.

While most bioluminescence systems seem to have their basic reaction of a luciferin that is oxidized with help of a luciferase, the similarities pretty much end there as the luciferin molecules involved are different, the luciferases are different and also often the cofactors involved are different. For example, to allow cypridinid luciferin to emit light no additional cofactors are needed while for

D-luciferin to emit light often a co-factor by the name of Mg-ATP is needed while for D-luciferin the cofactor Mg-ATP is required. The idea that there are multiple independent origins for bioluminescence was already proposed by Haddock et al (2010), who hypothesized that bioluminescence has evolved at least 40 times but probably even more than 50 times in different organisms. However, when investigating a variety of the different luciferins on Earth individually, their evolutionary history becomes more intriguing.

The luciferin coelenterazine is by far the most widespread of all luciferins as it can be found in many different phyla. Therefore, it is also the most complicated group where many uncertainties still exist. Overall, it seems that between the different phyla that use coelenterazine convergent evolution prevails due to the many independent origins of bioluminescence. However, this is also not undisputed as there are claims of parallel evolution between *Cnidaria* and *Ctenophora* (Delroisse et al., 2021). Furthermore, some phyla seem drastically understudied such as *Protozoa*, *Polifera* and *Echinodermata*. Interestingly, in the literature there does seem the tendency of painting all phyla with the same convergent brush. A great example of this is that in Lau & Oakley (2020), it was mentioned that all the different phyla, or at least certain members of those phyla evolved convergently, while the relevant sources did not appear to be present.

Within phyla, the situation becomes slightly less complicated. First of all, *Porifera*, *Echinodermata* and *Protozoa* not enough evidence is present to date to give a decisive answer regarding their evolutionary history. Secondly, there is *Ctenophora* which is the odd one out as divergent evolution is the likely explanation. Lastly, in the remaining five phyla that use coelenterazine as their luciferin parallel evolution is the verdict.

This is similar to what was observed in D-luciferin, which is the most well-studied luciferin. D-luciferin occurs only in phylum, the *Arthropoda*. While, the evolutionary history of *Phengodini* is not yet fully worked out (Arnoldi et al., 2010), between other lineages such as fireflies and click beetles parallel evolution is determined with reasonable certainty (Fallon et al., 2018).

Similar to coelenterazine, the evolution of Cypridina luciferin is also quite challenging to assess, albeit for a different reason. The main issue is that the phylogeny, especially in regards to the genus Vargula is quite unclear, consequently it is sometimes referred to as a “catch-all” genus (Morin, 2019). Nevertheless, there is evidence that the *Ostracods* that use this luciferin had a bioluminescent common ancestor as their light-emitting system likely evolved only once, making this a case of divergent evolution (Morin, 2009). The others that make use of cypridinid luciferin are several species of fish. However, due to the recent discovery of “kleptoproteins” where not only the luciferin but also the luciferases of the ostracods is reused by the fish, more research will be needed (Bessho-Uehara et al., 2020). Even though, this mechanic has only been observed in one species of fish, *Parapriacanthus ransonneti*, it is impossible to tell how widespread this phenomenon actually is. Moreover, the moment that unrelated species start taking each other’s proteins, the classical types of evolution used in this thesis no longer apply.

Tetrapyrrole luciferin is the most atypical of all the investigated luciferins. Not only because this luciferin can only be found in two seemingly unrelated lineages, Dinoflagellates and Euphausiids, but also because the luciferin used by these organisms are extremely similar but not the same. Furthermore, no dietary connection has been established between the two lineages which creates ambiguity regarding their relationship, if there is one. However, it was determined that the luciferases between these groups were analogous, resulting in the verdict of convergent evolution (Waldenmaier et al., 2012). When merely looking at *Euphausiids*, it becomes clear that there is a severe gap in knowledge regarding their evolutionary history. On the contrary, divergent evolution is possibly correct in *Dinoflagellates*.

Lastly, there is fungal and bacterial luciferin. These two groups turned out to be quite similar in more than one way. Firstly, in both groups the emergence of bioluminescence can be traced back to a common ancestor, making divergent evolution the most likely mode of evolution. Moreover, the fashion in which these findings have occurred is remarkably similar where mainly one researcher, H. Ke in case of fungal bioluminescence and P. Dunlap for bacterial light emission takes matters into their own hands and followed by several publications come to the aforementioned conclusions. However, there are still several additional caveats that impede on the possibility to come up with unassailable conclusions.

First of all, in the found literature the terms convergent evolution and parallel evolution are often used interchangeably or even considered to be a special case of convergent evolution as in Lau & Oakley (2020). This is especially visible in papers considering the evolution of coelenterazine based bioluminescence, where on multiple occasions the definitions used in this thesis conflicts with the descriptions employed by other researchers (Bessho-Uehara et al., 2020; Thuesen et al., 2010; Lindgren et al., 2012). However, it should also be mentioned that the definitions implemented for these modes of evolution are not the “holy grail” as they do tend to lead to a grey area when it comes to determining when parallel evolution actually should be considered convergent evolution.

Secondly, the phenomenon of symbiosis between organisms is underrepresented in this thesis. The main reason for this choice is that bioluminescent symbiosis with for example bacteria is actually quite rare and is treated as a deviation from the standard situation (haddock et al., 2010). Moreover, it would lead to cluttered results due to coevolution, an example of which is the possible coevolution between bioluminescent fungi and light-emitting arthropods (Oliveira et al, 2013). However, due to a gap in knowledge regarding this topic, these collaborations of unrelated bioluminescent organisms are for now merely an interesting observation that cannot lead to valid conclusions.

Lastly, many luciferins seem extremely understudied when it comes to their evolutionary history. For example, D-luciferin is extensively analyzed when it comes to fireflies and click

beetles and their importance for developments in biomedical research, the railroad worms and starworms are severely underexposed. The same is observed in tetrapyrrole-based luciferins where hardly anything is known about the evolution of Euphausiids. Therefore, to allow for more substantiated conclusions in these instances, more research about the evolution of bioluminescence is required.

Conclusion

In conclusion, it can be stated that when the different luciferins are compared between each other, convergent evolution prevails. However, the evolutionary history within each luciferin-based system is less well-defined. In coelenterazine and D-luciferin based bioluminescent systems, parallel evolution is observed most frequently, while in tetrapyrrole and cypridina luciferin convergent evolution still prevails. Moreover, it was determined that light emission in bacteria and fungi could in both cases be traced back to a common ancestor, making it an example of divergent evolution. Overall, this literature research aims to provide insight into the existing knowledge of the evolutionary history of bioluminescence and reveal underexposed aspects that require more extensive research.

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