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LIST OF PARTICIPANTS

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RESEARCH TOPICS

Topics proposed by participants

- Catalogue of studies of caffeine effects in fruit flies (DB)
- Circadian plasticity in fruit files - catalogue of studies (DB)
- Locomotor activity of wild-type and transgenic fruit files (DB)
- Sexual conflict in invertebrates – research evidence (JP)
- Influence of abiotic environment on invertebrates - research evidence (JP)
- Effects of Covid-19 on male fertility – current research field (SH)
- Research evidence on the relationship between human microbiome and asthma (SH)
- From conflict to coexistence in understanding wildlife interactions (SB)
- The role of human perception in shaping the mitigation strategies in wildlife conservation – map of the research field (SB)
- Gender bias among authors in biological studies (SAM)
- Temporal research trends in pollinator research (SAM)
- Microbiome studies in insects – map of research evidence (SAM)
- Effects of rapamycin on aging across animals (ES)
- The relationship between wing bit frequency and wing loading in insects (ES)
- Temperature-size rule and cell size in invertebrates (ES)
- What long-term experimental evolution studies are carried out (JP)
- How are studies on the association between opioids and risk of suicide carried out? (SH)
- Transition from narrative to systematic reviews in the last years (SB)

Topics selected by participants

- Gender bias among authors in biological studies (SAM)
- Temporal research trends in pollinator research (SAM)
- What long-term experimental evolution studies are carried out (JP)

Topics finally developed by participants

- Is gender parity still elusive among ecologists? - A systematic review
- Catalogue of long-term experimental evolution papers in certain selection regimes done on *Drosophila melanogaster*
- Temporal research popularity in bee pollinators during the last 25 years - do the contemporary problems and scientific effort match?

Is gender parity still elusive among ecologists? - A systematic review

by

Sayantani Basak and Debarati Bhattacharya

Is gender parity still elusive among ecologists? - A systematic review

Sayantani Basak and Debarati Bhattacharya

1. Aim of study

There is a gradual decline concerning women's participation in research globally. Women are actively pursuing bachelor's and master's degrees representing 53% of graduates, but their numbers drop off abruptly at PhD level. The absence of women from the highest echelons of science, publications and related decision-making is surprising, given the progress towards gender parity observed at all levels of education in recent decades. Research and publications are complementary for a successful career in science and the drive to publish in high prestige journals is an obvious response.

Given such impetus, success rates for elite journals are low – for instance, in Nature, less than 10 percent of submissions make it into print. Some studies have argued that gender parity analyses have not taken into account the prestige of the publication outlet. For instance, one explanation for these previous results could be that women publish less on average but focus more on higher prestige journals. In this respect, not every publication can be counted equally, as there are structural differences between publication types.

To probe deeper into this gender publication gap, we conducted a systematic review to identify the gap in publications in a prestigious journal, Nature. An easily accessible and objective indicator for the successful integration of women in science is the quantification of their scholastic activity as represented by "authorship" in scientific publications.

Thus, the study aims to systematically review gender parity in the fields of ecology by identifying the gender representation in *Nature* publication records globally in the last five years.

- Research Questions

In many research areas including life sciences, the position in an author list is important for reasons unrelated to the article's content, namely, prestige and eligibility for research grants. In these research areas, it is common practice that the first author indicates the person whose work underlies the paper as a whole, whereas the last or correspondence authorship suggests a person whose work or role made the study possible. However, certain studies have also demonstrated that in many countries, articles with women in key author positions receive fewer citations than those with men in the same positions. In light of these issues, we selected Nature journal to base our study for a period of five years between 2015 and 2019. We would address the following questions.

a. What is the percentage of the gender gap in Nature publication record as first and/or correspondence authors in the last five years in the field of ecology?
b. How is the nature of temporal dynamics and difference in female representation as first and/or correspondence author?
c. Are there gender-specific differences observed in productivity (number of visual objects), the affiliation of authors and consequent citation rates?

We formulated the following hypotheses which would help us to answer the research questions.

- Hypotheses (H)
    a. We predict that women would not be underrepresented as first or correspondence authors (H1).
    b. We expect a gradual increase in female representation as first or correspondence authors over time (H2).
    c. We expect women will have articles with increased affiliations and citations over time (H3).

2. Scope of the study

Element	Evidence
Population	Articles published in *Nature* journal (both first and correspondence).
Exposure	Categorize articles over time (2015-2019).
Comparators	Change over time in the number of publications and citations among the genders
Outcomes	[Not applicable]

3. Search-string

a. Web of Science
PUBLICATION NAME: (nature) *AND* **TOPIC:** (ecol*)
Refined by: DOCUMENT TYPES: (ARTICLE OR REVIEW) **Timespan:** 2015-2019.

b. Scopus
(**TITLE-ABS-KEY** (ecol*) AND **ISSN** (0028-0836)) AND **PUBYEAR** > 2014 AND **PUBYEAR** < 2020 AND (**LIMIT-TO** (DOCTYPE , "ar") OR **LIMIT-TO** (DOCTYPE , "re"))

4. Inclusion criteria for the studies

The inclusion criteria were set to be a period of 5 years between 2015 to 2019. We focused only on research articles and reviews and excluded letters, editorial materials, news items, short survey and note.

The search generated **124** studies in Web of Science and **178** studies in Scopus.

The overlaps were analyzed, and duplicates were removed. The final generated articles were **193.**

5. Protocol for data collection from the full texts

We would collect data to identify gender parity by determining gender representation ratio as first or corresponding author, increased gender parity over time, research citations (to be compared within each year and between genders) and number of affiliations of authors (among genders).

Thus, we would look for (a) year of publication (b) article type (c) gender of first author (d) gender of correspondence author (e) number of figures in the paper (f) number of citations (g) number of affiliations of first author (h) number of affiliations of correspondence author.

The data collection would be done under the following heads-

Sl No.	Year	Article Type	Title	Gender of first author	Gender of correspondence author	No. of visual objects in the paper	Citations	No. of affiliations of first author	No. of affiliations of correspondence author

Is gender parity still elusive among ecologists? - A systematic review

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Summary
Representation of women in science drops substantially at each career stage, from early student to senior investigator. Authorship can be used as an objective tool to determine successful integration and representation of women in scientific discourse. The position of an author in a paper and its citations helps to infer the author's contributions along with further spread and recognition of research. In this review, to determine whether gender parity is observed within the field of ecology, we gathered author's information from 146 articles published in *Nature* journal between 2015 and 2019. Despite recent progress, the gender gap appears likely to persist especially predominantly in authorship positions. Women are under-represented (~25%) as first and corresponding (~20%) authors on average. Affiliations of corresponding authors have gradually increased implying growing collaboration rates with a few exceptions. However, the citation rates continued to fluctuate throughout the years. Our findings show the implicit biases in key authorship positions which portrays stagnancy and inequitable academic environment specially in ecology. We emphasise on institutional support and consciousness of the asymmetries to strike a gender balance in academia.

Keywords: gender gap, *Nature*, ecology, systematic review, citations, affiliations

1. Introduction

There is a gradual decline concerning women's participation in research globally. Women are actively pursuing bachelor's and master's degrees representing 53% of graduates, but their numbers drop off abruptly at PhD level [1]. Yet the absence of women from the highest levels of science, publications and related decision-making is surprising, given the progress towards gender parity observed at all levels of education in the recent decade [2]. Research and publications are complementary for a successful career in science and the drive to publish in high prestige journals is an obvious response. However, it has been widely demonstrated that the representation of women as authors on scholarly high impact publications varies significantly [3].

In many research areas including ecology, the position in an author list is important not only from the article's content [4] but also for establishing scientific hold. In research areas, it is common practice that the first author indicates the person whose work underlies the paper as a whole, whereas the last or correspondence authorship suggests a person whose work or role made the study possible [5]. Authorship, therefore, constitutes as an identity to connotate research works. Thus an easily accessible

yet objective indicator for the successful integration of women in science could be the quantification of their scholastic activity as represented by authorship in scientific publications [4]. Yet in a study examining the proportion of women authorships in papers published from 1990-2011 across 21 science and humanities disciplines, popular ecology journal *ecology and evolution* had the seventh lowest proportion of women authors (22.76%) [6].

Certain studies have also demonstrated that articles with women in key author positions receive fewer citations than those with men in the same positions [4,7]. Male authors in the top 20% in career impact receive 36% more citations than their female counterparts [8]. Disparities are not limited to citations but are also highlighted in research affiliations. Studies reported that women are also less likely to participate in collaborations that lead to publication and are thus, much less likely to be listed as either first or corresponding author on a paper [7,9]. Therefore, women also experience a longer time to secure a faculty position in scientific fields such as ecology [7].

Some studies, on the other hand, have counter-argued that gender parity analyses have not taken into account the prestige of the publication outlet. For example, success rates for renowned journals are low – for example, in Nature, less than 10 percent of submissions make it into print [10].
Thus, the study aims to systematically review gender parity in the fields of ecology by identifying the gender representation in *Nature* publication records globally in the last five years, to address the question of gender symmetry. The following research questions (RQ) will be addressed.

a. What is the nature of temporal dynamics and difference in female representation as first and/or correspondence author in the last five years in the field of ecology?
b. Is there a gender gap in *Nature* publication records as the first and/or correspondence authors?
c. What is the rate of change in the affiliation and consequent citation rates among female authors over time?

2. Methodology

The initial protocol for the foremost steps of a systematic review was developed with the help of PECO (Populations, Exposure, Control, and Outcomes) statement [11] (table 1).

Table 1. PECO statement used to develop the study

Element	**Evidence**
Population	Authors of research articles, reviews or letters published in *Nature* journal in 2015-2019
Exposure	NA
Comparators	Female vs male authors
Outcomes	- number of articles published as a first author - number of articles published as a corresponding author - number of author affiliations - number of article citations

The systematic review was then conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [12] (figure 1).

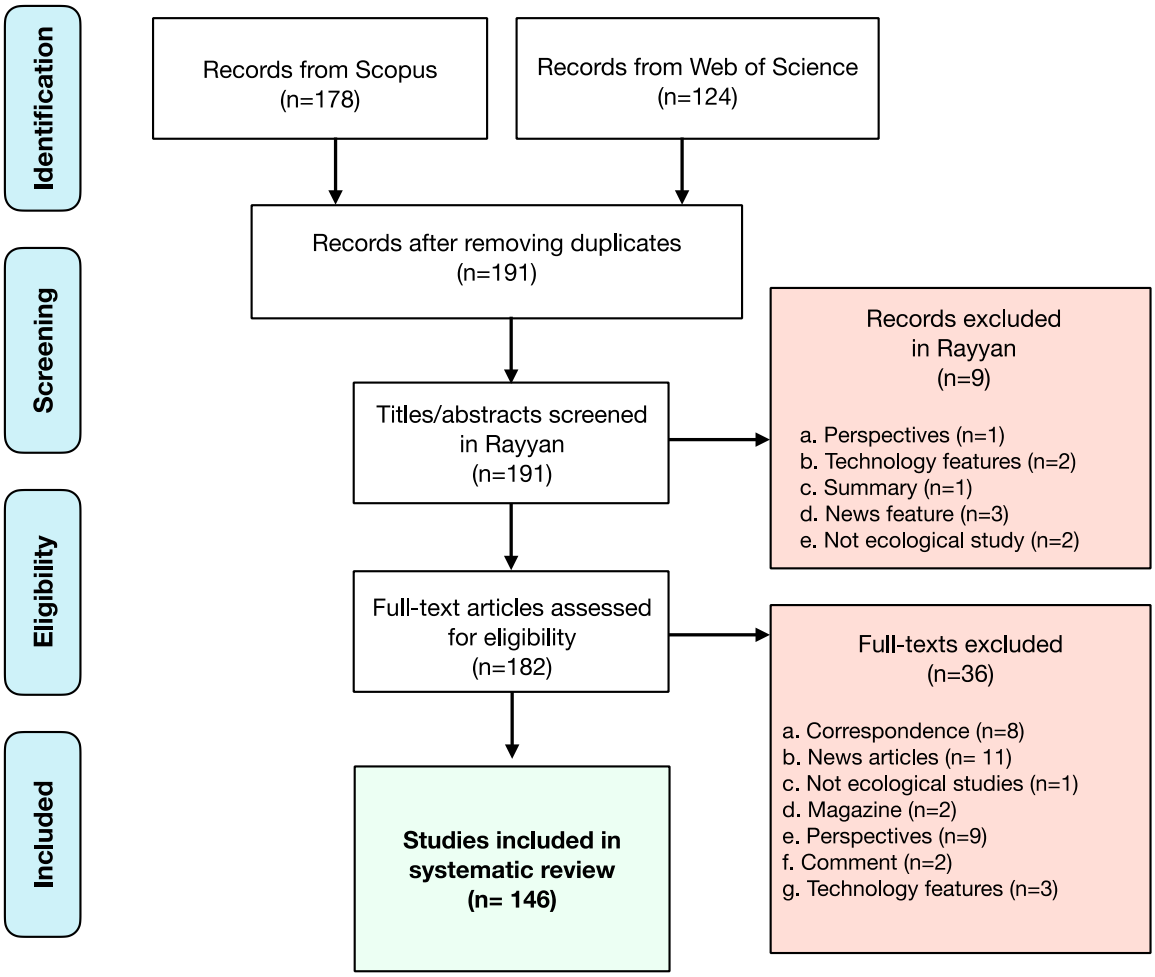

Figure 1. PRISMA flow diagram showing the procedure applied after the literature search (i.e. abstract and full-text screening against inclusion and exclusion criteria, and final included studies for systematic review).

2.1 Selection approach and eligibility criteria

The literature was queried in Scopus and Web of Science, which is considered as one of the main sources of citation data [13], for relevant research work published from January 1, 2015, to December 31, 2019. The search was conducted in June 2020 using the search terms Publication Name= (nature) AND Topic= (ecol*) AND Timespan: (2015-2019). The set of inclusion criteria were

i. The literature should be published in *Nature* journal.
ii. The selected research work should be published within the time frame (2015-2019).
iii. Literature should be limited to journal articles, review and letters.
iv. The published literature should address ecological studies.

We excluded editorial materials, news items, correspondences, short survey and notes from our final analysis.

2.2 Data extraction and quality assessment

The initial steps after removing duplicates returned 191 published articles from both Scopus (178 articles) and Web of Science (124 articles). The bibliography was also verified manually to eliminate any false positives which are usually more detrimental than false negatives in a systematic review [14]. Two authors (S.B. and D.B.), then independently screened abstracts in Rayyan [15] which enabled rapid exploration and filtration of studies supporting inclusion criteria eliminating chances of missing studies [16]. Full texts were thereafter assessed for eligibility and finally, the number of potentially included

articles was 146. The list of articles used in the review has been included in the supplementary materials with detailed bibliography (see supplementary material).

2.3 Data collection protocol and analysis

The data entry was done in three stages. First, a cursory review of publications identifying the gender of the first and correspondence author was undertaken. Determination of author gender was done by searching for images of an individual's name using the Google search engine in popular scientific domains (ResearchGate and Google Scholar). Additionally, gender searches were narrowed by including the name of the research institution or research subject among the search words where images were not identified (see supplementary material). Furthermore, help from popular social media (Twitter, YouTube and Facebook) was undertaken. This enabled to assign gender for 98.6% of papers (n=144) examined. The two case where gender could not be identified, the major cause was the absence of a photograph on the web.

Secondly, we searched for the number of citations for each article. Citations were noted from Scopus as it was primarily created for bibliographic and citation searching [17].
Finally, the first and corresponding author's affiliations in the study were noted for further analysis. Thus, for each database, we recorded the year of publication, the gender of the first and corresponding author (along with links to profile determining the gender), number of citations and number of affiliations of first and corresponding authors. The analysis for each entry averaged approximately 15 min since each record needed manual inspection to confirm the gender, affiliations and citations.
To compare significant differences among genders, we used linear model and p-value was adjusted by the Benjamini-Hochberg procedure. The data analyses were performed in R (version 3.6.1) using *tidyverse* package.

3. Results

We identified 146 studies that were included in our study to identify the pattern of gender symmetry comprising of 28 (in 2015), 32 (in 2016), 33 (in 2017), 34 (in 2018) and 19 (in 2019) articles in consecutive years. Out of the 146 published articles, there were 99 journal letters (67.8%), 33 research articles (22.6%) and 14 review articles (9.6%) respectively. There were two incidences where the gender of the first author could not be determined. This contributed to 98.6% of success rate in the author's gender determination.

i. Temporal dynamics in female representation (RQ1)

The overall temporal trend (figure 2a) showed the under-representation of women in publication record compared to male counterparts. The female representation was highest in 2015 for both first (n=10) and corresponding authors (n=9). Thereafter there was a gradual decline of female representation. With respect to female first authors, the numbers remained quite low throughout the subsequent years with only 6 (2016), 6 (2017), 5 (2018) and 5 (2019) authors representation. Corresponding female author representation was also low with 3 (2016), 4 (2017), 6 (2018) and 4 (2019) authors.

ii. Gender gap in publication record as first and/or correspondence authors (RQ2)

The results portrayed a wide disparity with the mean female representation as first authors being 21.61% (±7.51) and as corresponding authors being 16.85% ((±8.91) over the period of five years. The coefficient of variation for female as first and corresponding authors is 2.86 and 1.90 respectively. This indicated that there was little or no variation among female author representation over the years. Upon pairwise comparison between authors, significant gender difference was observed for both first authors ($p= 0.03$) and corresponding authors ($p= 0.01$).

iii. Temporal changes in the affiliation and citation rates (RQ3)

Affiliation for both female first and corresponding author had seen a drop in 2016 with 0.48 and 0.11 rate of fall (figure 2b). The rate gradually improved for female first authors to 1.25 and drastically for female corresponding authors to 2.69 in 2017. However, affiliations for female first authors continued to drop in subsequent years.

A more fluctuating trend for citation rates was observed (figure 2c). Citation rate for female first-authored articles had observed an increase in 2016 (1.75) and in 2018 (1.40) compared to its previous years, as opposed to intermediate declines in 2017 (0.68) and 2019 (0.94). The female corresponding trendline for citation rates was almost similar to the female first-authored trend, the only exception being in 2019 when the trend saw a slight rise (1.80).

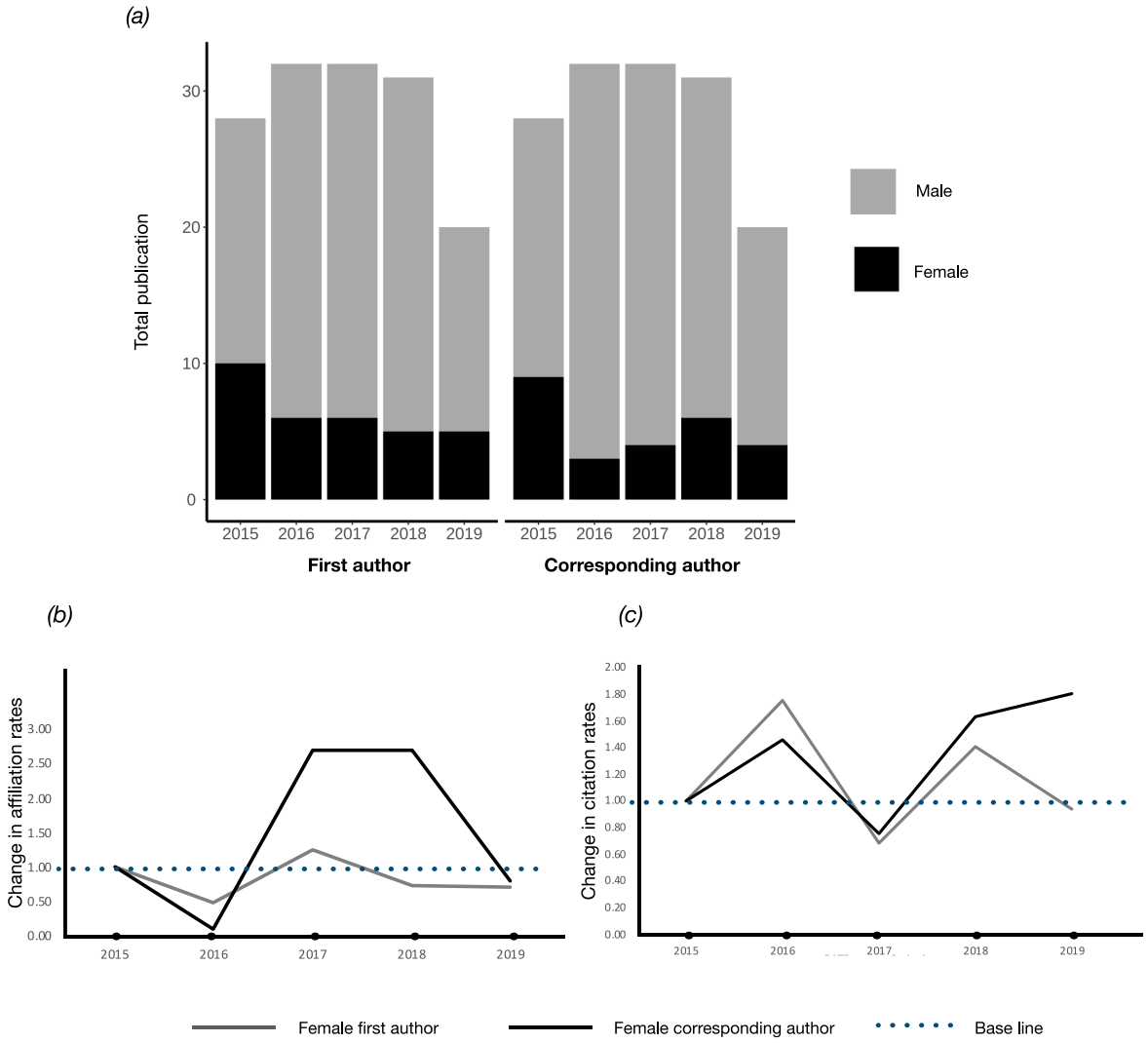

Figure 2. Gender gap representation *(a)* The temporal trend showing the representation of female and male author to the total number of articles published in the year. *(b)* Trend line indicating the rate of change of affiliation for female as the first and corresponding authored articles. *(c)* Trend line indicating the rate of change of citations for female as the first and corresponding authored articles.

4. Discussion

In scientific discourse, publishing is considered an imperative element for peer evaluation and scientific communication. Thus, the under-representation of women as authors in academic publications and in more prestigious journals potentially affects the representation of women faculty in academia. Our review provides a gender parity assessment of published articles from *Nature* from around the world for the field of research in ecology. Our analysis of 146 suitable studies indicated varied asymmetry

outcomes in gender balance overall in academic publishing. Our research showed that women representation in key authorship positions is stagnant and the academic publishing environment still remains inequitable. For instance, overall female author constitutes below 30% on average in the studied years with less than 25% representation as first authors. This result was quite intriguing given the fact that there has not been a significant drop in the total publication in the following years. In response, some studies have reasoned that as women are less likely to self-promote than men [18] and/or have a lower perception of their success [3], therefore may be more likely to fail to negotiate authorship position successfully [19]. As a consequence, we may definitely expect some lag between disparity in the first and last author positions, as it takes time for younger scholars to become leaders of research groups. Some studies said that that men and women are more likely to collaborate on papers with researchers of the same gender [3,20]. Our results indicate that despite of a rise in the number of affiliations for female first author in 2017, affiliations were nonetheless few. A rise in affiliations for female corresponding authors in later years is a good indicator of increasing collaboration. An interesting pattern was observed concerning citations of publications with female as key authors. Many previous studies across various disciplines reported that female authors attract fewer citations than their male counterparts [21,22] and our results contribute further showing that there was not much increase in citation rates in following years. Rather the trend was quite fluctuating. This makes it is plausible to assume that the lack of women in leadership or collaboration positions causes this accentuated female under-representation and resultant fewer citations.

We acknowledge some limitations in our study, which suggest caution in interpreting the data. The most important limitation study is the lack of information regarding academic rank and degree (e.g. doctoral vs post-doctoral degree) of the author and employment status which could be worthwhile to report co-relating with citation records.

However, there is no doubt that the one domain in which network science could offer insight is in the study of gender differences in authorship and collaboration patterns [23]. The path to gender balance in academia must involve not only through institutional support [24] but also through the consciousness of the asymmetries in the current collaboration network. This will ensure overcoming the career dichotomy between the two genders.

Authors' contributions. Both authors contributed equally.

Competing interests. We declare no competing interests.

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Reviewer: Mariusz Cichoń

This is an interesting contribution focusing on the inequality in a share of research articles published by woman and man in the *Nature* journal. The main finding shows the existence of such inequality with papers authored by male researchers being overrepresented in comparison to papers authored by female researchers. Interestingly, such inequality can be seen also in the citation number of the papers. It is quite intriguing as citing authors usually do not know the gender of the authors of the cited papers.

I am not an expert neither in systematic reviews nor in sociology of science, so the issues raised below may not be adequate, but the role of the reviewer is to point on some weak parts of the manuscripts. So, I will try.

First, I do not understand why the authors focused on papers published in Nature. I understand this is the top journal in life science, but how one can be sure that this journal is representative for other journals. I would understand if the authors focus on papers published in Biology Letters as such data could have some practical meaning for the publishing strategy of Biology Letters. Alternatively, one could understand publishing this data in Nature.

Second, the authors make little use of statistics in this review. Some statements seem to be based on simple comparisons by eye, not rigorous testing of hypotheses. For example, the authors discuss some temporal dynamics in number of papers or citations without formal tests. We do not know whether the observed changes are meaningful or just come by chance. Talking about statistics, it is not enough to report p-value as a report of the statistical test. One needs to report also some statistics and degrees of freedom if the parametric test is reported.

If it comes to methodology, I wonder what the authors did with cases in which there were many authors among which there were women and men, for example a case in which first authors was let's say a man and the corresponding a women. It is not clear to me if there were repeated entries of the same paper and if so how the authors dealt with repetitions.

One more remark. Why the authors expect a parity in the share of scientific output? Do you believe the parity would allow faster progress? Would you discard an important scientific discovery only because it was done by a man or woman? I believe the discoveries do not have gender!

Reviewer: Joanna Palka

Authors provide information about gender bias in ecological studies, based on Nature articles since 2015- 2019. This is a systematic review which has not been done before, it is a novel approach towards gender parity. In overall I would recommend this paper for publication after considering my suggestions and resubmission with proper corrections.

The structure of the paper was clear, introductory part explained quite well why the study is important. The goals of this publication were defined and subsequently accomplished both in methods as well as in the results section. For such a short time, authors managed well with data collection and data analysis provided together with interesting discussion.

Although, I would recommend some more work to make this paper even more clear. At the beginning of each paragraph, the tabulation should be included. Authors should explain why they choose ecology as a field of interest, is it because of the low proportion of females in the mentioned study? At line 130, the significance of differences can be verified after analysis, not before. Also, R as software should be properly cited. It would be good to write the used model explicitly. I do not understand what are the units used in Figure 2 b and c.

Reviewer: Sandra Åhlén Mulio

Overall, I liked you study very much! It is an interesting topic that certainly is of contemporary interest. I appreciate that you have a clear structure, aim and discuss limitations to your work. Your review is easy to follow and well written. Figures are clear and neat. Methods are clear and structured. I see no major flaw in hypothesis or statics used or presented. You have stayed within the word and element limit. You include a take home message, but I am however missing suggestions for future research or where you clearly see this research going in the future. I am also missing strong arguments or statements for why this is important as a study topic. I offer a few suggestions for improving your study below.

Summary
I would switch placed between the first and second sentence, some possible modifications needed to make it flow. Or, start with sentence about differences of men and women in broader terms of work/society. Working on the principle of a pyramid or a funnel, start wide and narrow it.

Line 19: "citations helps to infer the author's contributions", I am not sure if it is easy beyond a single author paper to infer their contributions based on its citation score? Maybe I am misunderstanding the aim of your sentence.
Line 26: "However, the citation rates continued to fluctuate throughout the years" This statement feels sudden or at least how it is formulated, maybe change into something like: However, results show that citation rates fluctuated through the years.
Line 28: especially instead of specially

Introduction
I would again start with a broader sentence, for example "The issue of gender difference and inequality which favors men has been prevalent throughout history and is still commonplace in today's society. From the political sphere and military power, to everyday life and hierarchy in the workplace. The field of science is no different…"

Line 40: I am not sure it is a response; publishing is a condition or a requirement even for funding, and thus tied to aims of publishing in high impact journals to prove you do good and important science so you can more easily get funded.
Line 44: content? Do you mean contribution to the content? A bit unclear, consider clarifying
Line 48: Thus,
Line 50: Yet,
Line 54: Omit Certain, and just start the sentence with Studies…..
Line 55-56: "Male authors in the top 20% in career impact receive ", what is meant by career impact? Is it a journal? In such case not italicized as previous examples.
Line 59-60: So because of lack of collaboration, they take longer to secure a faculty position? Or do you mean that in summary, with all previous listed reasons that they take longer to secure a faculty position?

Line 63: acceptance rates rather than success maybe to avoid confusion with the following words in your sentence.
Line 68: the study into this study, be specific that it is your interesting study!

Methodology
Line 94: which are, not is since it's plural databases you are referring to
Line 111: omit rapid, you are not trying to sell Rayyan as product ;)
Line 118: consider changing undertaken to performed or similar
Line 123: Same as previous
Line 124: cases, not case
Line 132: consider changing averaged approximately into "lasted/took approximately…"
Line 134: a linear model
Line 135: according to the Benjamini….instead of adjusted by the procedure.

Results
Line 144: remove 's on author, otherwise it sounds like the author was doing the gender determination when it was you who did :) If you mean to use the plural form just authors.
Line 147: publication records, plural
Line 151: authors representation into female author representation.
Line 157: Starting to sound like small discussion

Discussion
Line 185: female authorship
Line 188: "women are less likely to…"
Line 189: "therefore they may…"
Line 190: positions
Line 190: Consider reformulating the sentence starting here, I am slightly confused by the lag and the way you incorporate age into your sentence.
Line 200-202: Could you elaborate, I am not sure I follow why this is the case
Line 202: resulting few citations

Is gender parity still elusive among ecologists? - A systematic review

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Summary
Representation of women in science drops substantially at each career stage, from early student to senior investigator. Authorship can be used as an objective tool to determine successful integration and representation of women in scientific discourse. The position of an author in a paper and its citations helps to infer the author's contributions along with further spread and recognition of research. In this review, to determine whether gender parity is observed within the field of ecology, we gathered author's information from 146 articles published in *Nature* journal between 2015 and 2019. Despite recent progress, the gender gap appears likely to persist especially predominantly in authorship positions. Women are under-represented (~25%) as first and corresponding (~20%) authors on average. Affiliations of corresponding authors have gradually increased implying growing collaboration rates with a few exceptions. However, the citation rates continued to fluctuate throughout the years. Our findings show the implicit biases in key authorship positions which portrays stagnancy and inequitable academic environment specially in ecology. We emphasise on institutional support and consciousness of the asymmetries to strike a gender balance in academia.

Keywords: gender gap, *Nature*, ecology, systematic review, citations, affiliations

1. Introduction

There is a gradual decline concerning women's participation in research globally. Women are actively pursuing bachelor's and master's degrees representing 53% of graduates, but their numbers drop off abruptly at PhD level [1]. Yet the absence of women from the highest levels of science, publications and related decision-making is surprising, given the progress towards gender parity observed at all levels of education in the recent decade [2]. Research and publications are complementary for a successful career in science and the drive to publish in high prestige journals is an obvious response. However, it has been widely demonstrated that the representation of women as authors on scholarly high impact publications varies significantly [3].

In many research areas including ecology, the position in an author list is important not only from the article's content [4] but also for establishing scientific hold. In research areas, it is common practice that the first author indicates the person whose work underlies the paper as a whole, whereas the last or correspondence authorship suggests a person whose work or role made the study possible [5]. Authorship, therefore, constitutes as an identity to connotate research works. Thus an easily accessible

yet objective indicator for the successful integration of women in science could be the quantification of their scholastic activity as represented by authorship in scientific publications [4]. Yet in a study examining the proportion of women authorships in papers published from 1990-2011 across 21 science and humanities disciplines, popular ecology journal *ecology and evolution* had the seventh lowest proportion of women authors (22.76%) [6].

Certain studies have also demonstrated that articles with women in key author positions receive fewer citations than those with men in the same positions [4,7]. Male authors in the top 20% in career impact receive 36% more citations than their female counterparts [8]. Disparities are not limited to citations but are also highlighted in research affiliations. Studies reported that women are also less likely to participate in collaborations that lead to publication and are thus, much less likely to be listed as either first or corresponding author on a paper [7,9]. Therefore, women also experience a longer time to secure a faculty position in scientific fields such as ecology [7].

Some studies, on the other hand, have counter-argued that gender parity analyses have not taken into account the prestige of the publication outlet. For example, success rates for renowned journals are low – for example, in Nature, less than 10 percent of submissions make it into print [10].
Thus, our study aims to systematically review gender parity in the fields of ecology by identifying the gender representation in *Nature* publication records globally in the last five years, to address the question of gender symmetry. The choice of the journal is important for three reasons. Firstly, *Nature* is one of the high ranking prestigious (general) journal which has a strong effect on professional visibility and future research. Secondly, Success rate in *Nature* is very low (<10%) providing an opportunity to identify temporal representation of ecological studies. Finally, field specific journal (may) receive fewer citations catering to a specific sub field. The following research questions (RQ) will be addressed.

a) What is the nature of temporal dynamics and difference in female representation as first and/or correspondence author in the last five years in the field of ecology?
b) Is there a gender gap in *Nature* publication records as the first and/or correspondence authors?
c) What is the rate of change in the affiliation and consequent citation rates among female authors over time?

2. Methodology

The initial protocol for the foremost steps of a systematic review was developed with the help of PECO (Populations, Exposure, Control, and Outcomes) statement [11] (table 1).

Table 1. PECO statement used to develop the study

Element	**Evidence**
Population	Authors of research articles, reviews or letters published in *Nature* journal in 2015-2019
Exposure	NA
Comparators	Female vs male authors
Outcomes	- number of articles published as a first author - number of articles published as a corresponding author - number of author affiliations - number of article citations

The systematic review was then conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [12] (figure 1).

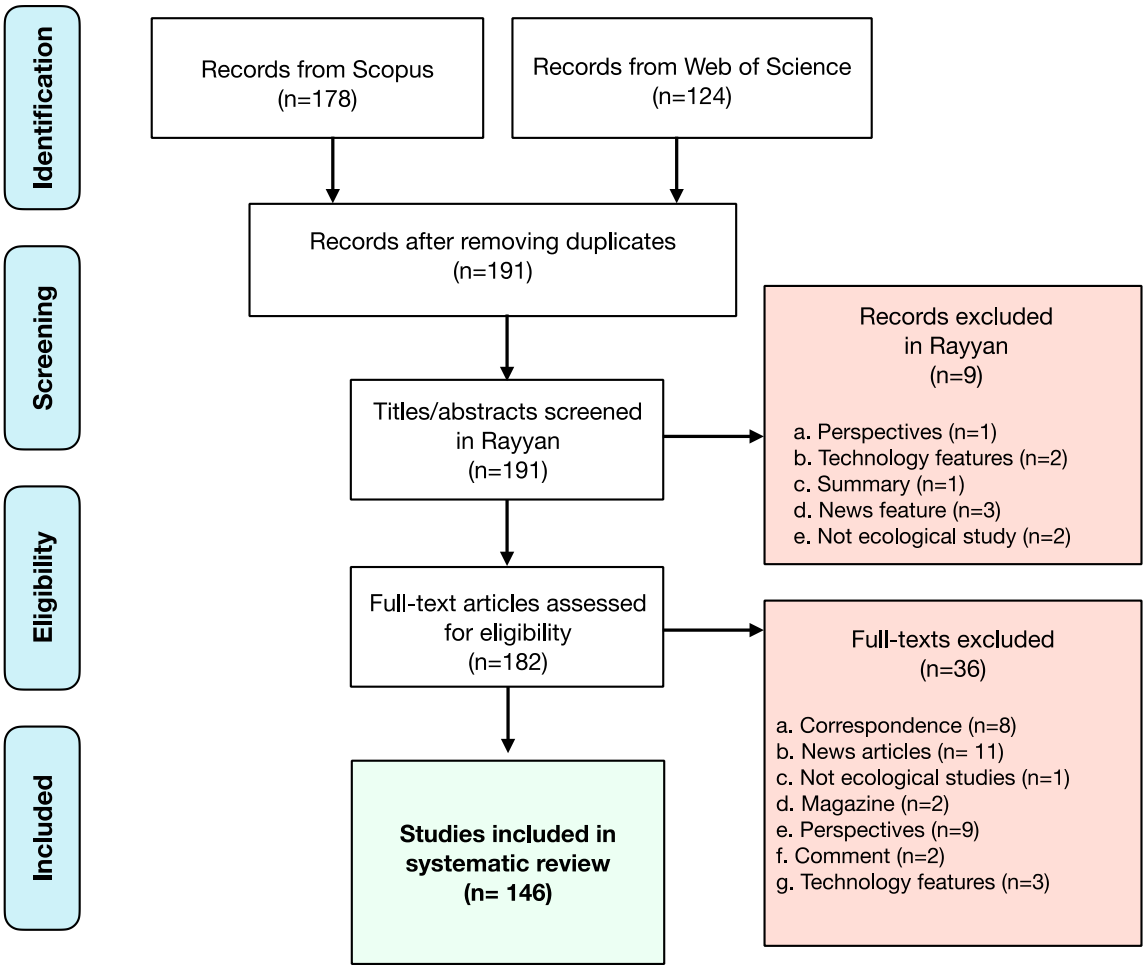

Figure 1. PRISMA flow diagram showing the procedure applied after the literature search (i.e. abstract and full-text screening against inclusion and exclusion criteria, and final included studies for systematic review).

2.1 Selection approach and eligibility criteria

The literature was queried in Scopus and Web of Science, which are considered as one of the main sources of citation data [13], for relevant research work published from January 1, 2015, to December 31, 2019. The search was conducted in June 2020 using the search terms Publication Name= (nature) AND Topic= (ecol*) AND Timespan: (2015-2019). The set of inclusion criteria were

v. The literature should be published in *Nature* journal.
vi. The selected research work should be published within the time frame (2015-2019).
vii. Literature should be limited to journal articles, review and letters.
viii. The published literature should address ecological studies.

We excluded editorial materials, news items, correspondences, short survey and notes from our final analysis.

2.4 Data extraction and quality assessment

The initial steps after removing duplicates returned 191 published articles from both Scopus (178 articles) and Web of Science (124 articles). The bibliography was also verified manually to eliminate any false positives which are usually more detrimental than false negatives in a systematic review [14].

Two authors (S.B. and D.B.), then independently screened abstracts in Rayyan [15] which enabled exploration and filtration of studies supporting inclusion criteria eliminating chances of missing studies [16]. Full texts were thereafter assessed for eligibility and finally, the number of potentially included articles was 146. The list of articles used in the review has been included in the supplementary materials with detailed bibliography (see supplementary material).

2.5 Data collection protocol and analysis

The data entry was done in three stages. First, a cursory review of publications identifying the gender of the first and correspondence author was performed. Determination of author gender was done by searching for images of an individual's name using the Google search engine in popular scientific domains (ResearchGate and Google Scholar). Additionally, gender searches were narrowed by including the name of the research institution or research subject among the search words where images were not identified (see supplementary material). Furthermore, help from popular social media (Twitter, YouTube and Facebook) was undertaken. This enabled to assign gender for 98.6% of papers (n=144) examined. The two cases where gender could not be identified, the major cause was the absence of a photograph on the web.

Secondly, we searched for the number of citations for each article. Citations were noted from Scopus as it was primarily created for bibliographic and citation searching [17].
Finally, the first and corresponding author's affiliations in the study were noted for further analysis. Thus, for each database, we recorded the year of publication, the gender of the first and corresponding author (along with links to profile determining the gender), number of citations and number of affiliations of first and corresponding authors. The analysis for each entry lasted approximately 15 min since each record needed manual inspection to confirm the gender, affiliations and citations.
To compare among genders, we used a linear model and p-value was adjusted using Benjamini-Hochberg procedure. The data analyses were performed in R (version 3.6.1) using *tidyverse* package [18].

3. Results

We identified 146 studies that were included in our study to identify the pattern of gender symmetry comprising of 28 (in 2015), 32 (in 2016), 33 (in 2017), 34 (in 2018) and 19 (in 2019) articles in consecutive years. Out of the 146 published articles, there were 99 journal letters (67.8%), 33 research articles (22.6%) and 14 review articles (9.6%) respectively. There were two incidences where the gender of the first author could not be determined. This contributed to 98.6% of success rate in the author gender determination.

iv. Temporal dynamics in female representation (RQ1)

The overall temporal trend (figure 2a) showed the under-representation of women in publication records compared to male counterparts. The female representation was highest in 2015 for both first (n=10) and corresponding authors (n=9). Thereafter there was a gradual decline of female representation. With respect to female first authors, the numbers remained quite low throughout the subsequent years with only 6 (2016), 6 (2017), 5 (2018) and 5 (2019) authors representation. Corresponding female author representation was also low with 3 (2016), 4 (2017), 6 (2018) and 4 (2019) authors.

v. Gender gap in publication record as first and/or correspondence authors (RQ2)

The results portrayed a wide disparity with the mean female representation as first authors being 21.61% (Standard deviation ±7.51) and as corresponding authors being 16.85% (Standard deviation ±8.91) over the period of five years. The coefficient of variation for female as first and corresponding authors is 2.86 and 1.90 respectively. This indicated that there was little or no variation among female author

representation over the years. Upon pairwise comparison between authors, significant gender difference was observed for both first authors (p= 0.03) and corresponding authors (p= 0.01).

vi. Temporal changes in the affiliation and citation rates (RQ3)

Number of affiliations for both female first and corresponding author had seen a drop in 2016 with 48% and 11% rate of fall (figure 2b). The rate gradually improved for female first authors to 125% and drastically for female corresponding authors to 269% in 2017. However, affiliations for female first authors continued to drop in subsequent years.

A more fluctuating trend for citation rates was observed (figure 2c). Citation rate for female first-authored articles had observed an increase in 2016 (175%) and in 2018 (140%) compared to its previous years, as opposed to intermediate declines in 2017 (68%) and 2019 (94%). The female corresponding trendline for citation rates was almost similar to the female first-authored trend, the only exception being in 2019 when the trend saw a slight rise.

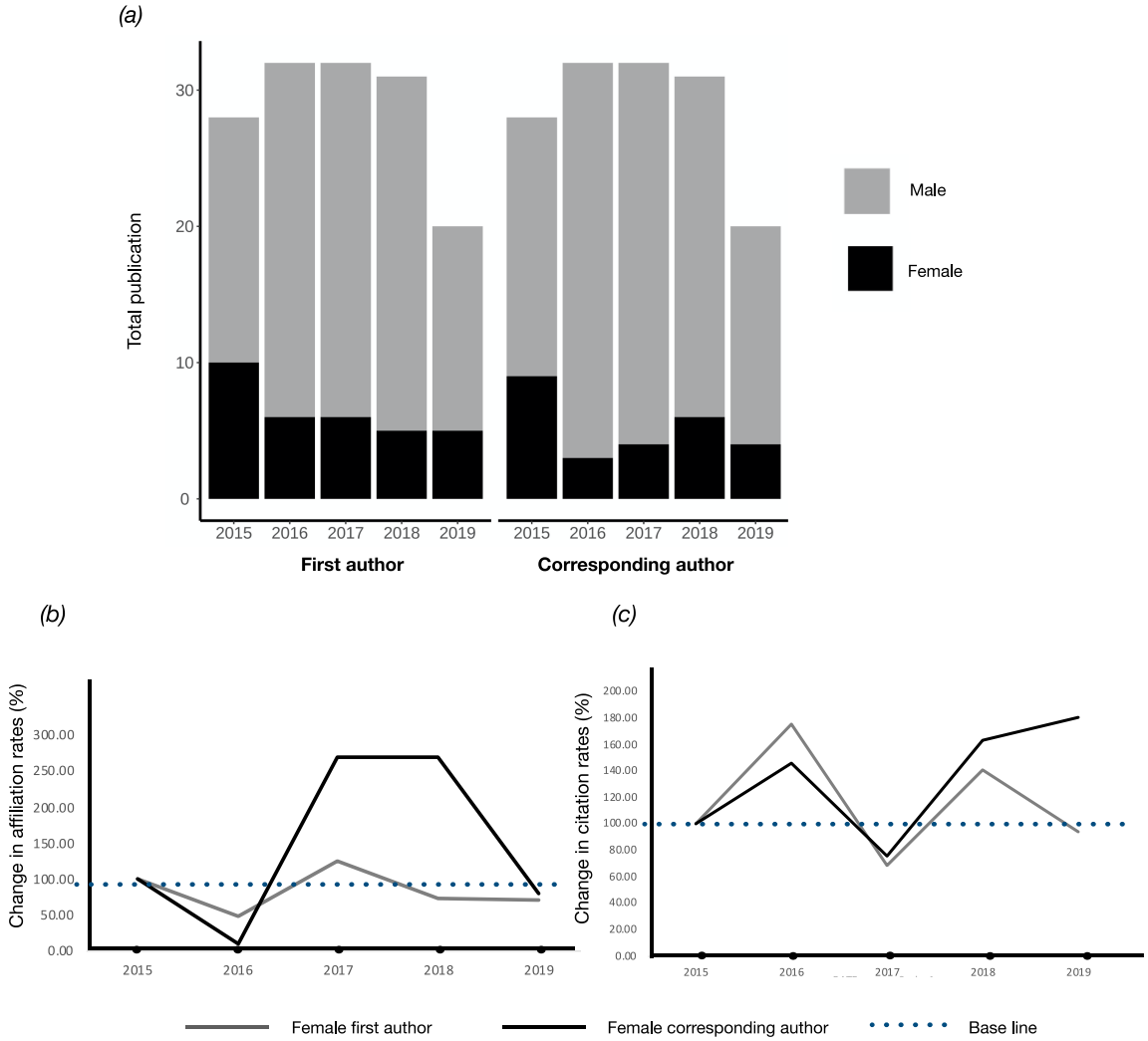

Figure 2. Gender gap representation *(a)* The temporal trend showing the representation of female and male author to the total number of articles published in the year. *(b)* Trend line indicating the rate of change of number of affiliations for female as the first and corresponding authored articles. *(c)* Trend line indicating the rate of change of citations for female as the first and corresponding authored articles.

4. Discussion

In scientific discourse, publishing is considered an imperative element for peer evaluation and scientific communication. Thus, the under-representation of women as authors in academic publications and in

more prestigious journals potentially affects the representation of women faculty in academia. Our review provides a gender parity assessment of published articles from *Nature* from around the world for the field of research in ecology. Our analysis of 146 suitable studies indicated varied asymmetry outcomes in gender balance overall in academic publishing. Our research showed that women representation in key authorship positions is stagnant and the academic publishing environment still remains inequitable. For instance, overall female author constitutes below 30% on average in the studied years with less than 25% representation as first authors. This result was quite intriguing given the fact that there has not been a significant drop in the total publication in the following years. In response, some studies have reasoned that as women are less likely to self-promote than men [19] and/or have a lower perception of their success [3], therefore may be more likely to fail to negotiate authorship position successfully [20]. As a consequence, we may definitely expect some lag between disparity in the first and last author positions, as it takes time for younger scholars to become leaders of research groups. Some studies said that that men and women are more likely to collaborate on papers with researchers of the same gender [3,21]. Our results indicate that despite of a rise in the number of affiliations for female first author in 2017, affiliations were nonetheless few. A rise in affiliations for female corresponding authors in later years is a good indicator of increasing collaboration. An interesting pattern was observed concerning citations of publications with female as key authors. Many previous studies across various disciplines reported that female authors attract fewer citations than their male counterparts [22,23] and our results contribute further showing that there was not much increase in citation rates in following years. Rather the trend was quite fluctuating. This makes it is plausible to assume that the lack of women in leadership or collaboration positions causes this accentuated female under-representation and resultant fewer citations.

We acknowledge some limitations in our study, which suggest caution in interpreting the data. The most important limitation study is the lack of information regarding academic rank and degree (e.g. doctoral vs post-doctoral degree) of the author and employment status which could be worthwhile to report co-relating with citation records.
However, there is no doubt that the one domain in which network science could offer insight is in the study of gender differences in authorship and collaboration patterns [24]. The path to gender balance in academia must involve through institutional support [25]. We do believe that research or discoveries do not have gender, but it is important to have the consciousness of the asymmetries in the current collaboration network. This will ensure overcoming the career dichotomy between the two genders.

Authors' contributions. Both authors contributed equally.

Competing interests. We declare no competing interests.

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Catalogue of long-term experimental evolution papers in certain selection regimes done on *Drosophila melanogaster*

by

Ewa Szlachcic and Joanna K. Palka

Catalogue of long-term experimental evolution papers in certain selection regimes done on *Drosophila melanogaster*

Joanna K. Palka and Ewa Szlachcic

1. Aim of study

Experimental evolution allow to address and answer a wide range of important biological questions. Model organisms, like *Drosophila melanogaster* are often used in such studies, because of their features such as short generation time or small size, which allows to obtain many generations in relatively short time. In this study we want to examine what kind of different types of selection were performed on this species. We will provide information on the quantity of different types of artificial selection in relation to the number of evolved generations. Such research will help to identify gaps and will show possible future directions.

2. Detailed scope of the study

Element	**Evidence**	**Key-words**
Population	*Drosophila melanogaster*	Drosophila melanogaster OR "D. melanogaster" OR "fruit fl*"
Exposure	Long term experimental evolution	experiment* AND (evolution* OR selection*) AND ("long term" OR long-term OR *generation*)
Comparators		not applicable
Outcomes	All types of long-term artificial selection done on *Drosophila melanogaster*	

3. Search-string

Scopus - 293 document results

TITLE-ABS-KEY (experiment* W/3 (evolution* OR selection*) AND ("d. melanogaster" OR "Drosophila melanogaster" OR "fruit fl*")) AND ("long term" OR long-term OR *generation*)

Web of Science - 635 document results

TS=(experiment* AND (evolution* OR selection*) AND ("D. melanogaster" OR "Drosophila melanogaster" OR "fruit fl*") AND ("long term" OR "long-term" OR *generation*))

4. Inclusion criteria for the studies

Protocol for screening the abstract for eligibility

1. It the research is in English? No -> excluded
2. If the study is done on *Drosophila melanogaster*? No -> excluded
3. Is this paper experimental or is it review? Review -> excluded
4. Is it a research article? No -> excluded
5. If the paper is about experimental evolution? No -> excluded
6. If it is long-term/multi-generation research (above nine generation)? No -> excluded
7. Is it experimental evolution done in selection regime? No -> excluded
8. Is the selection regime explained? No -> excluded
9. Is it an artificial selection (not natural)? No -> excluded

The rest studies included for full-text screening.

5. Protocol for data collection from the full texts

Check if the criteria (especially number of generations and selection regime) are met.

Extraction of data of interest into separate document:

- title,
- year,
- selection regime,
- number of generations.

Catalogue of long-term experimental evolution papers in certain selection regimes done on *Drosophila melanogaster*

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Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University

Abstract

Experimental evolution is a powerful approach that provides an opportunity to study evolutionary processes in real-time. It gives the chance to define and control the level of environmental change by limiting it to one factor or any desired combination of factors. Thus, the adaptive response to specific environmental factors can be isolated and analysed. Papers about experimental evolution are growing in number with time. Model organisms are popular in such studies because of their short generation time and small size. Here we performed a systematic review to catalogue the long-term experimental evolution studies done on *Drosophila melanogaster*. The outcome of 113 published studies revealed the variety of different selection regimes. However, the main focus in experimental evolution was on physiological, environmental and sexual selection regimes, especially diet, resistance to starvation or desiccation and altered sex ratio. Most of the experimental evolution research was performed on animals evolved from 9 to 49 generations. Together with the growing number of generations, the number of studies is decreasing. Our study shows the trend in long-term experimental evolution. Identification of research gaps will provide insight into future possibilities of studies.

Keywords: experimental evolution, artificial selection, systematic review, fruit fly

Introduction

Experimental evolution is the study of evolutionary processes that happen in populations in response to conditions imposed by the experimenter [1]. Such experiments can define and control the degree of environmental change, limiting it to a single factor or any desired combination of factors [2]. It is a research framework that offers the opportunity to study evolutionary processes experimentally in real-time [3]. Because of its functionality and relatively easy performance, this type of research is common in biological studies. Wide range of questions can be answered in many areas of evolutionary biology. Starting from the selection of organisms to some environmental stresses like temperature [4,5] or nutrition [6,7] to a variety of physiological aspects, like sleep deprivation [8].

Model organisms are often used in such studies, because of their features such as short generation time or small size, which allows to obtain many generations in a relatively short time. For the last 100 years, *Drosophila Melanogaster* was used as a model organism [9]. There was a time, during which it was the most significant model organism, which led to the establishment of new scientific disciplines [10]. *D. melanogaster* is a species of small fly, often found in the vicinity of rotting fruits from which it earned a common name of a fruit fly or vinegar fly. This small, yet complex organism became one of the first model organism and it is still extensively studied. With time experimental evolution experiments are growing in numbers. That, in turn, translates into an increasing number of research articles. The immense variety of experiments performed on model organisms can be overwhelming, especially for a researcher which just started his/her career in that field.

To provide some insight into the world of long term selection experiments we decided to make a systematic review of studies done on the fruit flies. In this study, we want to examine what kind of different types of selection were performed on this species. This will tell us about the number of such experiments and thus about trends in research. We will also provide information on the number of evolved generations in each of the selection experiment. Our research will help to identify gaps and will show possible future directions of research.

Materials and methods

To start our research, we first defined the scope of our study by developing a PECO (Population, Exposure, Comparators and Outcomes) statement (Table 1). The literature search was conducted using the Web of Science database and Scopus database. The research focuses on all studies published up to June 2020. We searched for studies including a combination of the terms 'experiment*' close to either 'evolution*' or 'selection*' and 'D. melanogaster' or 'Drosophila melanogaster' or 'fruit fl*' and either 'long term' or 'long-term' or '*generation*' in the title, abstract and keywords. These databases searches retrieved a total of 675 references, from which 259 were duplicates. Among 416 articles, we looked for research that met all of the following criteria: publication in English, research article, study regarded *D. melanogaster* and experimental evolution longer than 9 generations in which flies stay under artificial selection in every generation.

Table 1. Long-term experimental evolution PECO (population, exposure, comparators and outcomes) statement

Element	**Evidence**	**Key-words**
Population	*Drosophila melanogaster*	Drosophila melanogaster OR 'D. melanogaster' OR 'fruit fl*'
Exposure	Long term experimental evolution	experiment* W/3 (evolution* OR selection*) AND ('long term' OR long-term OR *generation*)
Comparators		not applicable
Outcomes	All types of long-term artificial selection done on *Drosophila melanogaster*	

Established references were independently screened by us at the title and abstract level for relevance and eligibility using Rayyan QCRI (https://rayyan.qcri.org). Studies which did not met the evidence criteria were excluded. Conflicts were resolved through discussion and decisions were taken mutually. From 416 articles, 32.5% (135 publications) were included for full-text screening. Full texts of references were obtained using reference manager Zotero (Corporation for Digital Scholarship, Vienna, US) and by a manual search. Eight articles were excluded because of the lack of full text. During the full-text review, we checked whether the selection regime is explained and the number of generations is given. We rejected 14 papers which did not meet our criteria. During data collection, we extracted for each research selection regime and the number of generations of artificial selection. We coded the data to 6 categories of selection regimes, namely environmental conditions, morphology, physiology, genetics, sexual and other and 5 ranges for generations number. If the experiment has involved more

than one category, the dominant one was selected. Additionally, a short description of the selection regime was added to each response. Data extraction was managed with Google forms (Google LLC, Mountain View, US) provided in a Supplement S1. After a full-text review, 113 studies were considered relevant.

To get an insight into the frequency of regimes appearing in the research articles we performed a word cloud text analysis using the free online Wordcloud generator (https://www.wordclouds.com/). Short descriptions of each experiment were put together and analysed by category to which they were classified. Word cloud represents the frequency of words used to describe each of the selection regimes. The words were analysed separately for each category. To visualise our data we also created a heat map using R software, with ggplot2 function (R Core Team, 2018).

Results

During the full-text screening, each article was assigned to one (or more if more experiments were performed) of six determined categories. Here, we will briefly discuss the established categories (full list of obtained selected regimes is provided in Supplement S2). The environmental conditions category includes studies in which surrounding was altered e.g. temperature, diet, different larval density or presence of mould. The second category includes experiments which put pressure on changes in physiology, e.g. development rate, increased viability, resistance to desiccation, chronic nutritional stress or infections. The third category includes a selection for traits connected with sexual selection e.g. evolution in altered sex ratio, sexual conflict, mate competition or mate choice. Next category, morphology includes a selection for an exact body trait e.g. aristal branches, body melanisation, body size, bristle number. The genetic category is focused on the changes in the genome caused by e.g. inbreeding or male-limited X-chromosome evolution. If the experiment did not fit into any previously described categories, it was assigned into the "other" category. We have found only nine papers which were included in the last category, and they were focused for instance on learning, life history or geotaxis.

Fig. 1A. shows that in environmental studies, the diet was manipulated the most. Flies were also often kept in different oxygen concentration (hypoxia or hyperoxia) or in harsh temperatures. In terms of physiology (Fig. 2B), studies often were focused on the evolution of resistance to either starvation or desiccation. Sexual selection studies (Fig. 1C) often performed enforced monogamy or were altering sex ratio in which populations were evolving. When it comes to traits connected with morphology (Fig. 1D) most common was a selection for bristle number or secondly, for body size. Genetic studies (Fig. 1E) were quite evenly distributed, which is not surprising because there were only five such studies. A similar situation is observed in the last category (Fig. 1F), each of the studies had a different aim, but two of them were connected with life-history traits and another two with learning abilities.

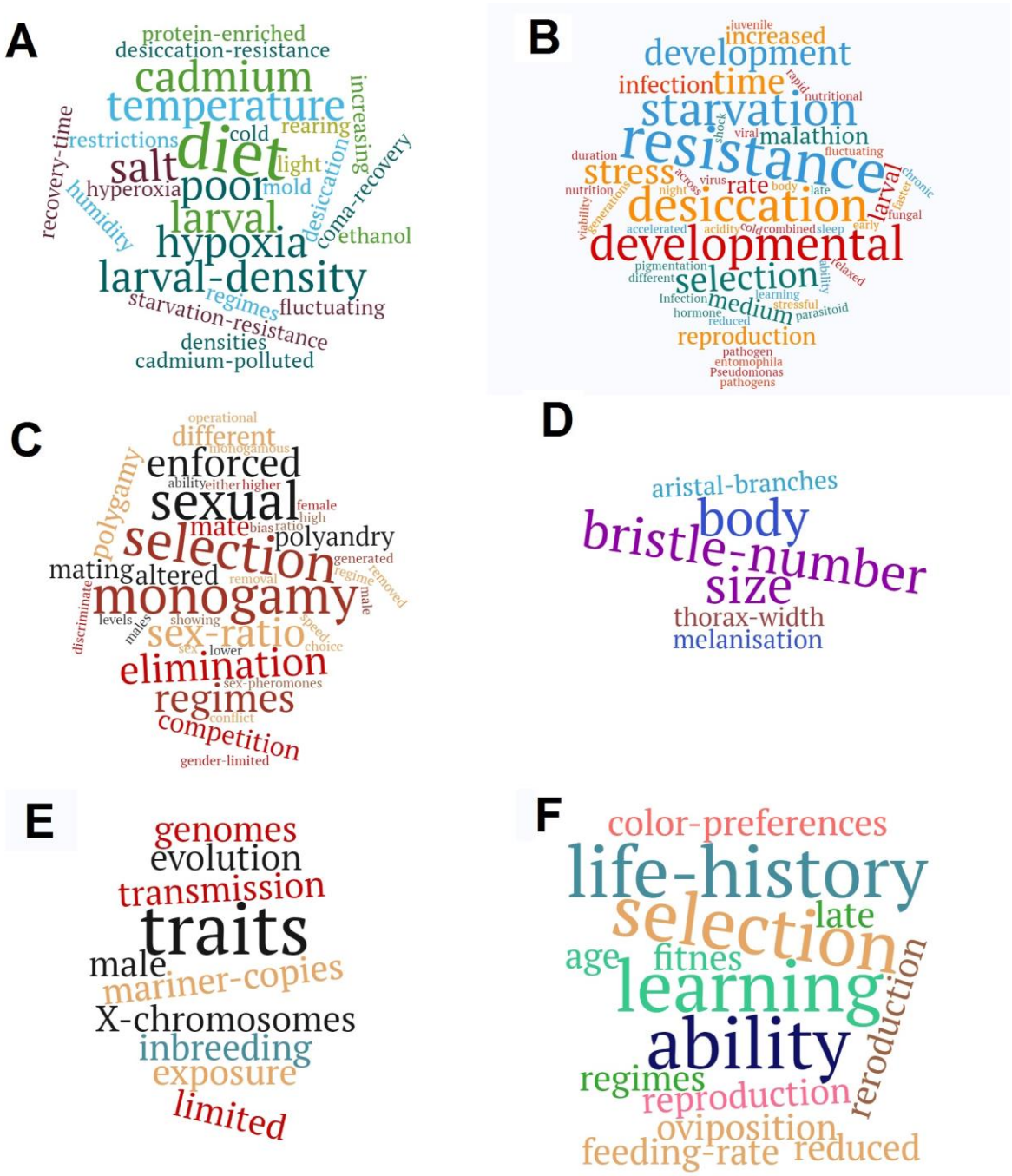

Fig. 1. Word cloud of six categories of selection regimes with specifications of the regime. A. Environmental conditions. B. Physiology. C. Sexual. D. Morphology. E. Genetics. F. Other. The word size represents the frequency of occurrence specific regime (separately for each category).

Additionally, we wanted to take a look at the number of studies performed in each category in relation to the number of evolved generations. The six categories of selection regime and five ranges of generations numbers serve us to form a heat map (Fig. 2.) with 30 cells in which each cell represents the number of studies.

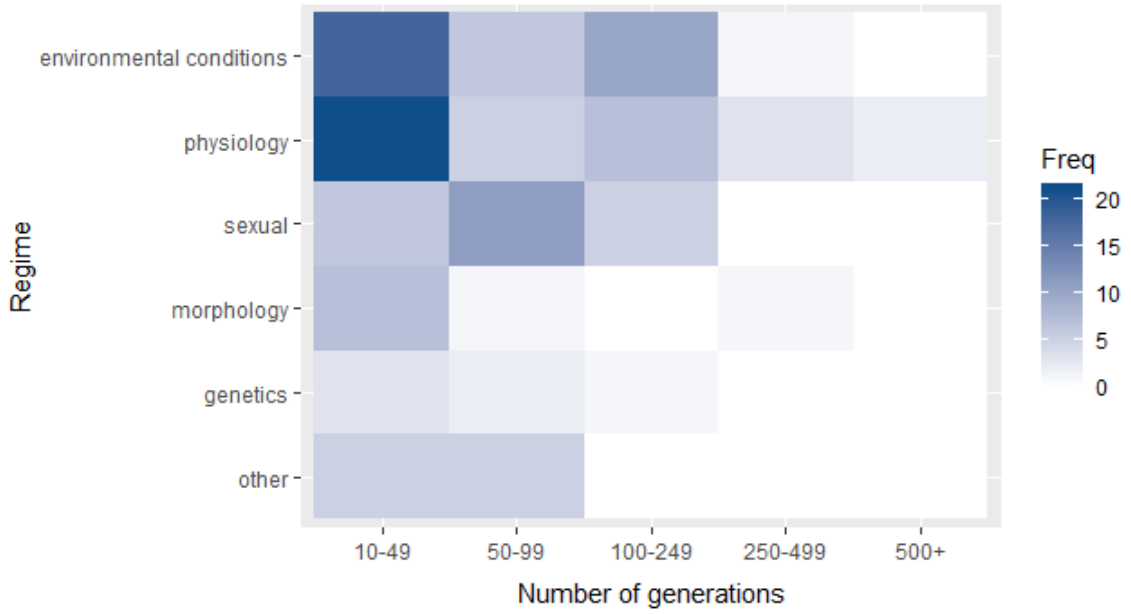

Fig. 2. Frequency of papers with different selection regimes (rows, 6 categories) in relation to the number of evolved generations (columns, 5 ranges). The frequency of papers is represented by color; darker colors represent a larger number of papers.

As we can see in Fig. 2. most of the experimental evolution studies were focused on physiological and environmental selection regimes. Sexual selection regimes situate on the third place in terms of their quantity. The first range of generation number (from 10 to 49 generations) was most common in almost all categories. Exception for this trend was found in sexual selection category, for which most popular was second range (from 50 to 99 generations) and in "other" category in which two first ranges were equally popular. In the third range (from 100 to 249 generations) we still observe some studies belonging mostly to the three most popular categories. Together with the growing number of generations, the number of studies is decreasing. Only eight studies can be found in the last two ranges of generation number. Two of them are longer than 500 generations and can be only found in the physiology category.

Discussion

In our study, we analysed long term experimental evolution papers with defined selection regime. We analysed 113 papers, with the oldest one published in 1952 and the newest in 2020. Majority of them was published after 2000 (97 papers), which shows the growing popularity of such studies in the last twenty years. Publications that we have found were mostly focused on the selection in different environmental regimes [e.g. 11–13] or for certain physiological traits [e.g. 14–16]. Also, experiments connected with evolution in different sexual selection regimes were quite popular. The reason for such an outcome may lay in the feasibility of manipulation of surroundings for example temperature, humidity or the presence of pathogens. Least popular were experiments connected with morphology or genetics, probably because this kind of selection is more time-consuming or more sophisticated equipment is needed. That may explain why they were also performed for a smaller number of generations. Another reason for the shortage of long term selection experiment (more than 250 generations) may lay in limited genetic variation. It is easy to imagine that variation for e.g. morphological trait is lost after dozens of generations (especially if the population size is small). Moreover, long term evolutionary studies are costly, so their conducting is limited by funding.

Reviewed studies revealed a variety of different selection regimes. Some of them, for instance, mouldy conditions [17], humidity [18], light [19], colour preferences for oviposition [20], mating speed [21] are mentioned only once among all 113 analysed publications. There are not many studies that are focusing on other categories, such as learning abilities or life-history traits, which in our opinion are also very interesting topics. On the other hand, we found that studies testing different dietary manipulations or resistance to various factors are quite common indicating that those fields of study are saturated. In conclusion, our catalogue of long-term experimental evolution papers shows trends and gaps in the experimental evolution research done on fruit flies until now. It provides insight into future possibilities of studies, especially what kind of selection regimes could be studied more. Our study can be a good starting point for a researcher who plans to start conducting experiments in this field.

However, our study has its limitations, even though we did our best to narrow their number as much as possible. Primary limitation of this study could be the subjectivity of the authors, which may lead to misunderstanding of the research topics and assigning them into the wrong category. Categories are artificially created by us for the purpose of this study, which does not mean that they are not overlapping in real life. Also, as mentioned in the Methods section, some studies were performed in complex experiments with a couple of selection pressures and only predominant selection was assigned into a category, which can cause some bias in our results. Moreover, it was often hard to decide on the category of particular study because the selection regime could be different than the secondary goal of the paper. In this case, research that distinguishes primary selection regime from the secondary outcome is needed.

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Reviewer: Szymon Drobniak

The study I was asked to review was focused on a systematic summary of experimental evolution experiments performed on Drosophila melanogaster as a model species in evolutionary biology. The study seems to assemble a comprehensive sample of published studies and does a good job in identifying such papers. However, there are some issues that may further improve the study and make it much more useful for the general audience.

1) Experimental evolution experiments are notoriously difficult for many reasons, one of which being the number of generations possible to obtain in a given analysis. The authors did a nice job summarizing generation numbers in the context of the types of analyzed traits. However, there are many other aspects of experimental evolution studies that would be interesting (and are easily obtainable as regularly reported in such studies). For example – the usual problem of such studies is the type of medium used – many labs have problems with this issue and would appreciate summarizing information for practical use in future procedure validations. Such information would also enable filling gaps in terms of which media are used with specific types of studied traits. Other parameters that would enrich the literature considerably would be: the origin of used flies (e.g. inbred stocks, wild flies, previous experiments); geographic locations and spatial distribution of studies; allowed lifespan of flies (i.e. whether they were allowed to age or not).
2) The categorization of studies seems a bit simplistic. It should be based on broad categories of studies rather than a mix of experimental treatments and traits measured (as far as I understand – some studies where categorised as "other" as looking at life-history traits, but could be as well put into another group based on the general study objective. At the moment the supplementary table suggests the categorization is a mix of experimental treatments and types of measured characters – better description would be needed, and maybe a 2x2 categorisation (treatment vs. trait type).
3) Although this is a systematic review – it would benefit from some simple statistics, e.g. summarizing the distribution of generation numbers vs. study types, by providing a simple statistical benchmark of whether there was (or not) a relationship between the number of generations and the study type.

Minor remarks:

- The key word clouds seem a bit chaotic, I would suggest organising them better and consider removing colouration (unless it has a meaning on itself)
- Introduction: it would be nice to provide an overview of the most significant findings and advances brought about by experimental evolution studies on D. melanogaster

Reviewer: Sylwia Herman

Evaluated review broaches one of the topical fields of contemporary science. The submitted manuscript presents a noteworthy subject of experimental evolution. It opens up new horizons in biology providing an opportunity for observation and understanding the evolutionary processes in real-time.

The abstract is clear and arises curiosity. Summary consisted of all necessary sections except for the methods used in systematic review preparation. Both the aim of publication and explanation of its

importance were substantially described. Such justification along with proper keywords inclusion made it suitable for the scientific paper.

The introduction is concise and contains all crucial issues to that realm of research. It also clearly elucidates complex concepts even for someone outside the given field of study. This section perfectly fits the scope of the whole paper. It contains sufficient clarification of review topic choice and justifies the importance of provided results.

The material and method section is greatly detailed. The PECO table seems to be filled properly and the time frame of searched articles is impressive. However, one of the suggestions is to add the words "long-dated" and "long-run" to the searched terms. The more synonyms are used, the higher probability of gathering all valid articles is. Another suggestion would be to put information about the number of found papers on a Prisma diagram. It will be more transparent and easier to visually assess. The profile of selection regimes presented in the table form is clear and understandable.

As far as the results section is concerned, reasonable decisions about methods of data analysis were made. They are well-organized and vastly interesting. Figure 1. states a creative, clear, and eye-catching way to show the categories of selection regimes and serves as an attractive variety. However, discussion about these categories at the beginning of the section does not bring anything new. Except for the last sentence, it is just a repetition of the supplementary table S2. Thereupon, this short discussion seems to be unnecessary.

The discussion section is well-prepared. For each result, at least a couple of arguments were proposed. The section division into paragraphs, with consecutive results justification, and gap recognition facilitates organizing the assimilation of information. The paragraph enumerating limitations of the carried-out analysis deserves appreciation for rationalism and honesty. It will be invaluable for future research and subsequent systematic reviews in the given subject.

In terms of the requirements of the Biology Letters Journal, the main ones were completely respected. The word number does not exceed the established limit as well as the number of figures and displayed elements.

In the whole paper, there is an adequate number of citations that are suitable for particular statements. The presence of some spelling mistakes or missing words is probably just a consequence of the limited time frame. Overall, however, the editing style was at a high level of scientific language.

In conclusion, the systematic review is written very well.

Reviewer: Debarati Bhattacharya

Comments-(Major)

In Abstract Line no 18 mention of word 'animal'. Why it is inappropriate because the authors were studying about Drosophila a fruit fly. Fruit flies are generally insects and is an ideal organism for the study of animal development. It truly does not fit as an animal.

Line number 14-Mention of 113 articles. But survey of the data entry in excel sheet revealed that the authors eliminated some articles due to not fitting some of their criteria. The data entry showed 'yes' for 114 article and 'no' for the remaining (115-136). Which means the number in the abstract of included articles must be 114.

Word Cloud- Fig (F) specifying the 'other' group shows the word reroduction. But the supplementary attachment showed the table where there is a mention of the word reproduction.

Data entry table in Excel showed the year 60s ,70s,80s,90s, and 2000. But in main report line number 137 in the discussion it is mentioned 1952**.** In excel the data entry is from 60s and they are 1962,1963,1967.

Article title Catalogue fits the authors generalization of the data into specific categories to make the research findings more meticulous for the readers.

Comments-(Minor)

Line 114- re-gime must be without hyphen.

Line 146- long term is without a hyphen it must be long-term.

Line 158-stu-dies is with hyphen it must be studies.

Short comments-

- Good Job in a short time
- Screening of 4 decades of papers is commendable (Data entry in Excel sheet)
- They did the meta-analysis well Fig1 and Fig2.
- Research gap was nicely highlighted in lines 152-154.

Catalogue of long-term experimental evolution papers in certain selection regimes done on *Drosophila melanogaster*

Joanna K. Palka, Ewa Szlachcic

Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Krakow, Poland

Abstract

Experimental evolution is a powerful approach that provides an opportunity to study evolutionary processes in real-time. It gives the chance to define and control the level of environmental change by limiting it to one factor or any desired combination of factors. Thus, the adaptive response to specific environmental factors can be isolated and analysed. Papers about experimental evolution are growing in number with time. Model organisms are popular in such studies because of their short generation time and small size. Here we performed a systematic review to catalogue the long-term experimental evolution studies done on *Drosophila melanogaster*. The outcome of 113 published studies revealed the variety of different selection regimes. However, the main focus in experimental evolution was on physiological, environmental and sexual selection regimes, especially diet, resistance to starvation or desiccation and altered sex ratio. Most of the experimental evolution research was performed on animals evolved from 9 to 49 generations. Together with the growing number of generations, the number of studies is decreasing. Our study shows the trend in long-term experimental evolution. Identification of research gaps will provide insight into future possibilities of studies.

Keywords: experimental evolution, artificial selection, systematic review, fruit fly

Introduction

Experimental evolution is the study of evolutionary processes that happen in populations in response to conditions imposed by the experimenter [1]. Such experiments can define and control the degree of environmental change, limiting it to a single factor or any desired combination of factors [2]. It is a research framework that offers the opportunity to study evolutionary processes experimentally in real-time [3]. Because of its functionality and relatively easy performance, this type of research is common in biological studies. Wide range of questions can be answered in many areas of evolutionary biology. Starting from the selection of organisms to some environmental stresses like temperature [4,5] or nutrition [6,7] to a variety of physiological aspects, like sleep deprivation [8]. With time experimental evolution experiments are growing in numbers. That, in turn, translates into an increasing number of research articles.

Model organisms are often used in such studies, because of their features such as short generation time or small size, which allows to obtain many generations in a relatively short time. For the last 100 years, *Drosophila Melanogaster* was used as a model organism [9]. There was a time, during which it was the most significant model organism, which led to the establishment of new scientific disciplines [10]. *D. melanogaster* is a species of small fly, often found in the vicinity of rotting fruits from which it earned a common name of a fruit fly or vinegar fly. This small organism contributed to many most significant findings in evolutionary biology. Thomas Morgan had redefined the theory of inheritance using *Drosophila* to establish that genes were found within chromosome [11]. Using Drosophila in the 1920s, Muller discovered that x-rays caused an enormous increase in the mutation rate of genes, which could lead to chromosome breakage [12]. For the past decades, it has become a predominant model used to

understand how genes direct the development of an embryo to a mature multicellular organism [10]. This animal became one of the first model organisms and it is still extensively studied till today.

To provide some insight into the world of long-term selection experiments we decided to make a systematic review of studies done on the fruit flies. In this study, we want to examine what kind of different types of selection were performed on this species. This will tell us about the number of such experiments and thus about trends in research. We will also provide information on the number of evolved generations in each of the selection experiment. Our research will help to identify gaps and will show possible future directions of research.

Materials and methods

To start our research, we first defined the scope of our study by developing a PECO (Population, Exposure, Comparators and Outcomes) statement (Table 1). The literature search was conducted using the Web of Science database and Scopus database. The research focuses on all studies published up to June 2020. We searched for studies including a combination of the terms 'experiment*' close to either 'evolution*' or 'selection*' and 'D. melanogaster' or 'Drosophila melanogaster' or 'fruit fl*' and either 'long term' or 'long-term' or '*generation*' in the title, abstract and keywords. These databases searches retrieved a total of 675 references, from which 259 were duplicates (Prisma diagram provided in a Supplement S3). Among 416 articles, we looked for research that met all of the following criteria: publication in English, research article, study regarded *D. melanogaster* and experimental evolution longer than 9 generations in which flies stay under artificial selection in every generation.

Table 1. Long-term experimental evolution PECO (population, exposure, comparators and outcomes) statement

Element	**Evidence**	**Key-words**
Population	*Drosophila melanogaster*	Drosophila melanogaster OR 'D. melanogaster' OR 'fruit fl*'
Exposure	Long-term experimental evolution	experiment* W/3 (evolution* OR selection*) AND ('long term' OR long-term OR *generation*)
Comparators		not applicable
Outcomes	All types of long-term artificial selection done on *Drosophila melanogaster*	

Established references were independently screened by us at the title and abstract level for relevance and eligibility using Rayyan QCRI (https://rayyan.qcri.org). Studies which did not met the evidence criteria were excluded. Conflicts were resolved through discussion and decisions were taken mutually. From 416 articles, 32.5% (135 publications) were included for full-text screening. Full texts of references were obtained using reference manager Zotero (Corporation for Digital Scholarship, Vienna, US) and by a manual search. Eight articles were excluded because of the lack of full text. During the full-text review, we checked whether the selection regime is explained and the number of generations

is given. We rejected 14 papers which did not meet our criteria. During data collection, we extracted for each research selection regime and the number of generations of artificial selection. We coded the data to 6 categories of selection regimes, namely environmental conditions, morphology, physiology, genetics, sexual and other and 5 ranges for generations number. If the experiment has involved more than one category, the dominant one was selected. Additionally, a short description of the selection regime was added to each response. Data extraction was managed with Google forms (Google LLC, Mountain View, US) provided in a Supplement S1. After a full-text review, 113 studies were considered relevant.

To get an insight into the frequency of regimes appearing in the research articles we performed a word cloud text analysis using the free online Wordcloud generator (https://www.wordclouds.com/). Short descriptions of each experiment were put together and analysed by category to which they were classified. Word cloud represents the frequency of words used to describe each of the selection regimes. The words were analysed separately for each category. To visualise our data we also created a heat map using R software, with ggplot2 function (R Core Team, 2018).

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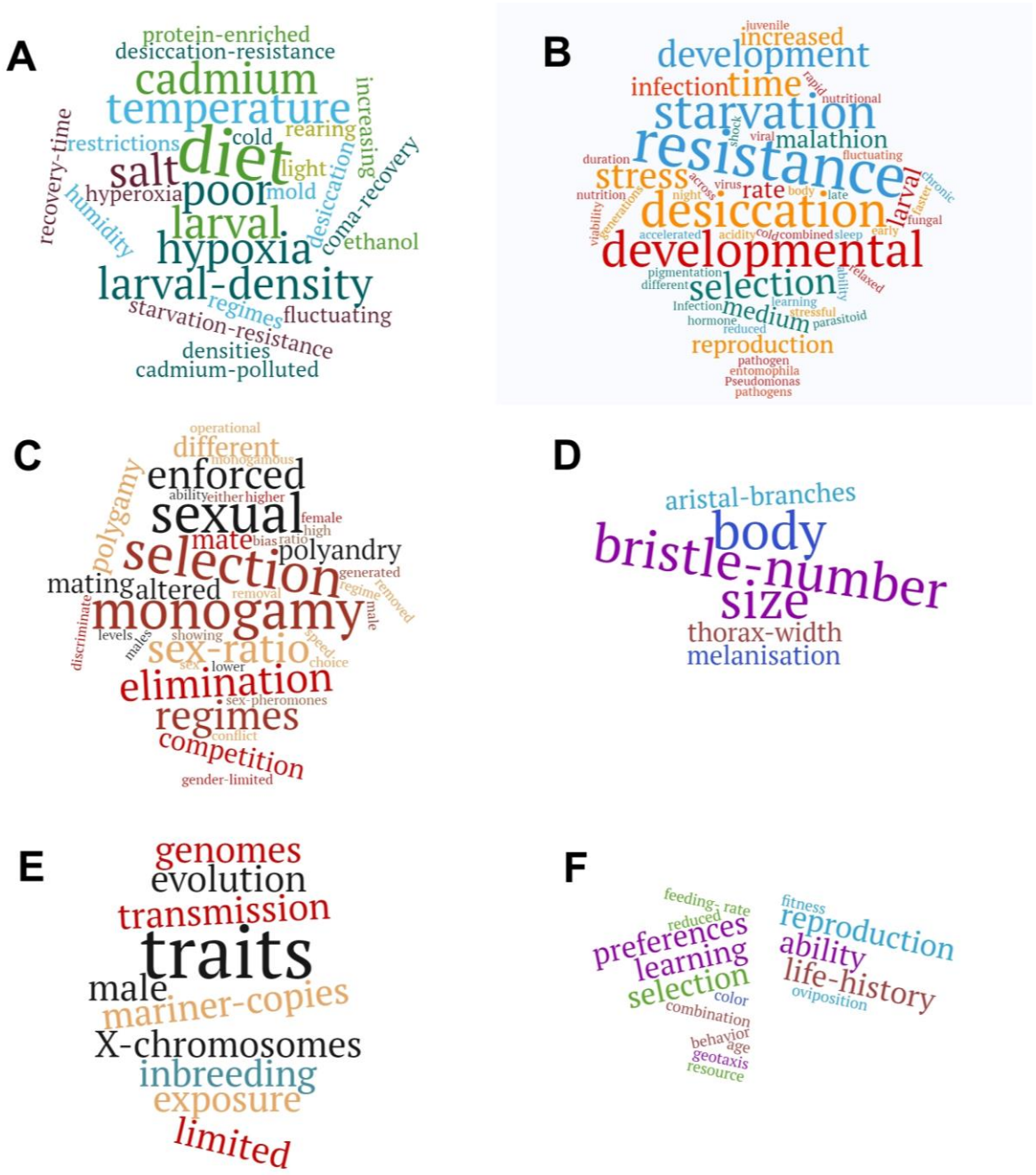

Fig. 1. Word cloud of six categories of selection regimes with specifications of the regime. A. Environmental conditions. B. Physiology. C. Sexual. D. Morphology. E. Genetics. F. Other. The word size represents the frequency of occurrence specific regime (separately for each category).

Fig. 1A. shows that in environmental studies, the diet was manipulated the most. Flies were also often kept in different oxygen concentration (hypoxia or hyperoxia) or in harsh temperatures. In terms of physiology (Fig. 2B), papers were mostly about the evolution of resistance to either starvation or desiccation. Sexual selection studies (Fig. 1C) often performed enforced monogamy or were altering sex ratio in which populations were evolving. When it comes to traits connected with morphology (Fig. 1D) most common in publications was a selection for bristle number or secondly, for body size. Genetic studies (Fig. 1E) were quite evenly distributed, which is not surprising because there were only five such studies. A similar situation is observed in the last category (Fig. 1F), each of the studies had a different aim, but two of them were connected with life-history traits and another two with learning abilities.

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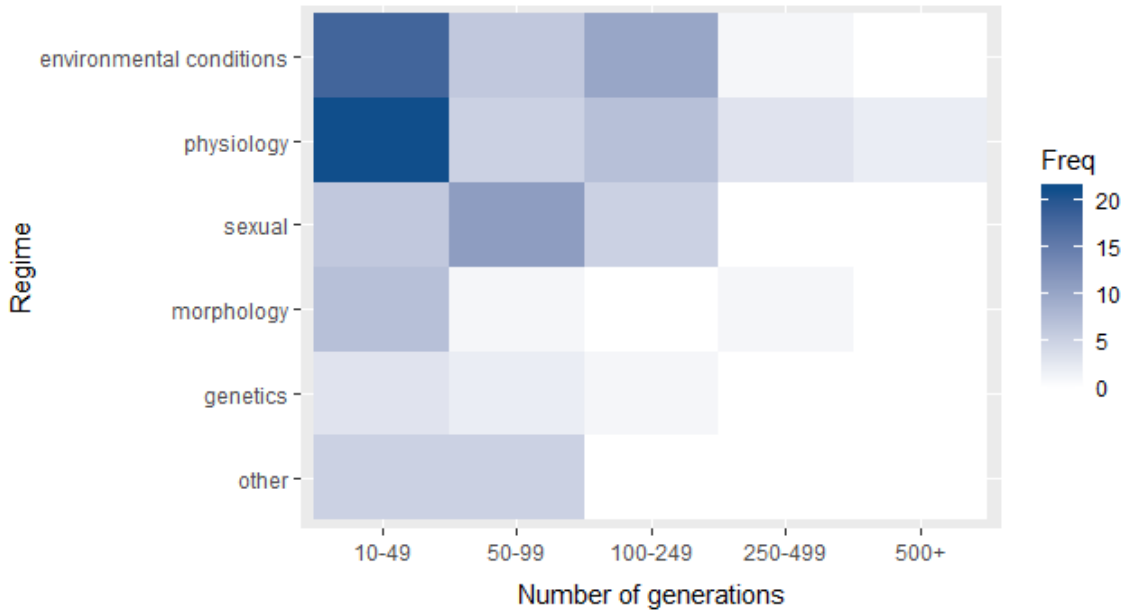

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However, our study has its limitations, even though we did our best to narrow their number as much as possible. The main issue of this study is rooted in the fact that one selection experiment could be published in multiple papers also with different generations time. This does not represent the true number of selection regimes conducted from the first generation. That is why, instead of such study we focused our research on a number of published papers, which seems more appropriate in this case. Another limitation could be the subjectivity of the authors, which may lead to a misunderstanding of the research topics and assigning them into the wrong category. Categories are artificially created by us for the purpose of this study, which does not mean that they are not overlapping in real life. Also, as mentioned in the Methods section, some studies were performed in complex experiments with a couple of selection pressures and the only predominant selection was assigned into a category, which can cause some bias in our results. Moreover, it was often hard to decide on the category of particular study because the selection regime could be different than the secondary goal of the paper. In this case, research that distinguishes primary selection regime from the secondary outcome is needed.

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Supplement S2.

Table S1. Classification of selection regimes extracted from screened literature

Selection regime	Specification
environmental conditions	oxygen level (hypoxia, hyperoxia), light (after evolution in darkness), different temperature, increasing temperature, fluctuating temperature, cold resistance, hot resistance, thermally stressful environment, diet (poor larval food e.g. diluted, poor in nutrition; protein-enriched diet), dietary restriction, feeding regimes, ethanol-supplemented food, decreased recovery time (cold resistance), rearing densities, larval density, cadmium-supplemented food, salt-supplemented food, temporally or spatially enriched food, mouldy conditions
physiology	chronic nutritional stress, desiccation resistance, developmental rate, developmental time, increased viability, juvenile hormone in the food, malathion resistance, night sleep duration, parasitoid resistance (*A. tabida*), pathogens and infection resistance, reproduction time, starvation resistance, stressful medium, viral infection, starvation combined with cold shock (fluctuating across generations)
morphology	aristal branches, body melanisation, body size, bristle number, thorax width
genetic	male-limited X-chromosome evolution, enforcing father-to-son transmission of the X-chromosome, male-limited genomes, inbreeding, marinere
sexual	sex ratio (male or female bias regime), sexual conflict, mate competition, elimination of sexual selection (enforced monogamous mating), mate choice, gender-limited selection, high sexual selection, mating speed, ability to discriminate sex pheromones
others	feeding rate, geotaxis, late age reproduction, learning ability, life-history selection regimes, colour preferences for oviposition, reduced fitness

Supplement S3.
Prisma diagram was created using Microsoft Power Point (Microsoft, Redmond, US).

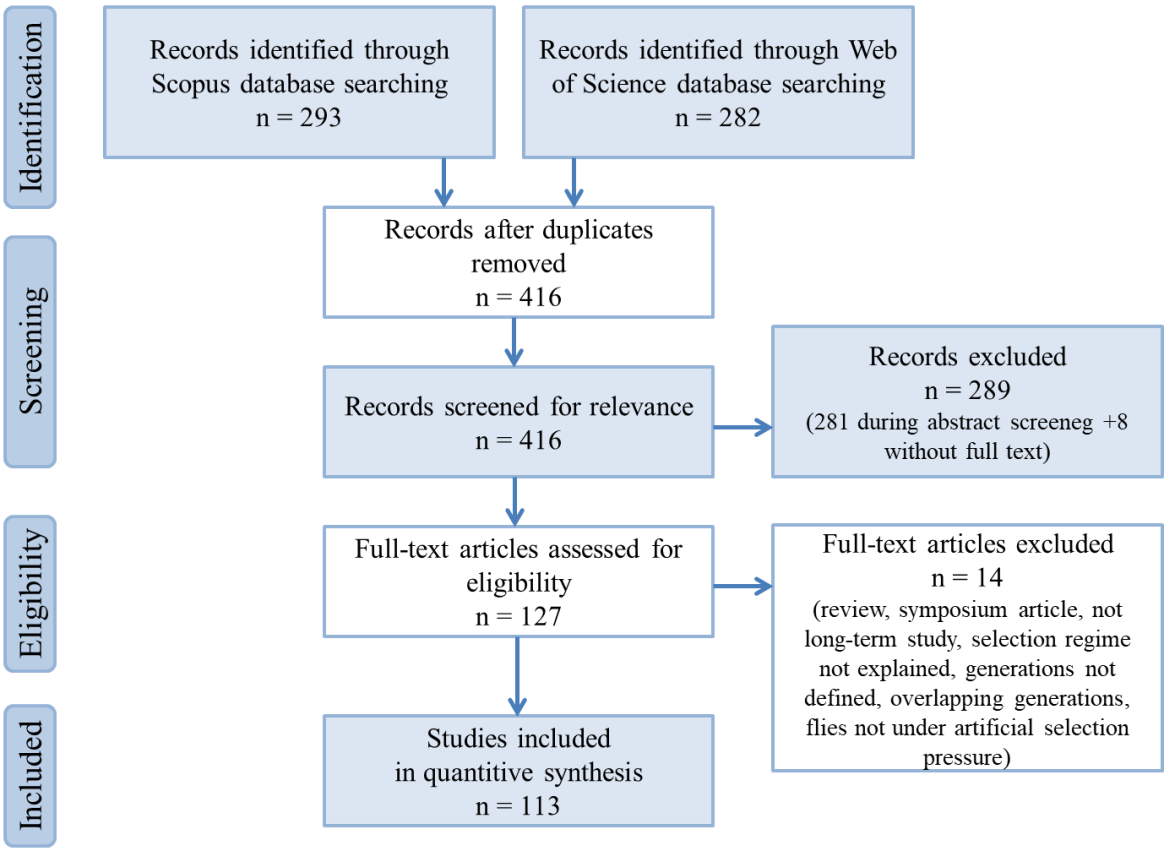
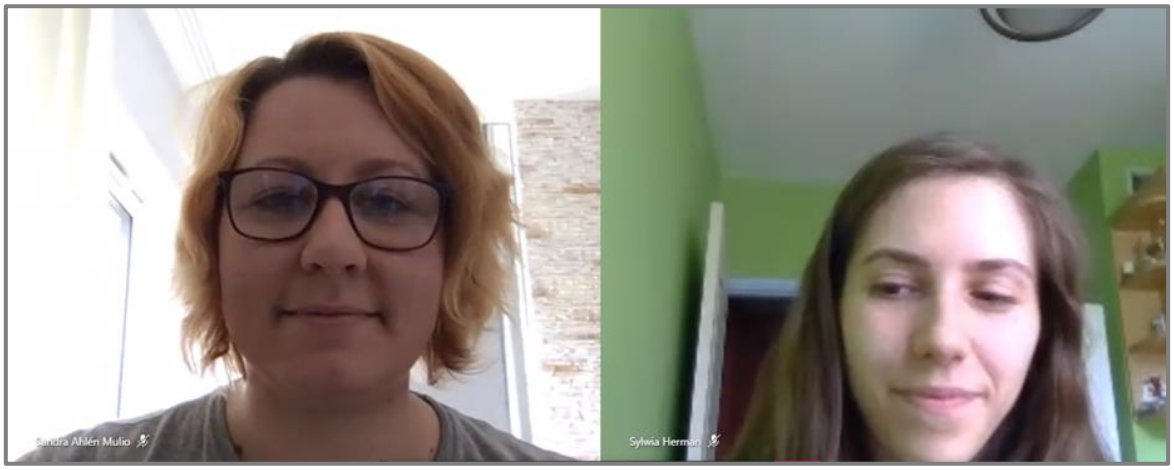


Fig. S1. Prisma flow diagram of systematic review of long-term experimental evolution done on fruit flies

Temporal research popularity in bee pollinators during the last 25 years - do the contemporary problems and scientific effort match?

by

Sandra Åhlén Mulio and Sylwia Herman



Temporal research popularity in bee pollinators during the last 25 years

Sandra Åhlén Mulio and Sylwia Herman

Aim and importance:

Studying the research on pollinators is important due to the rapid decline in abundance of pollinators in recent years. Pollinators carry out important ecosystem services such as pollination, production of resources such as honey and beeswax. Their decline would impose an immense threat to human food production and economic burden on the human society if pollination was to be done by hand or artificially. Thus, looking at the patterns and trends in pollinator research is important in order to establish that the research conducted is relevant to the modern questions and problems faced. Analysing the history of research is also useful in terms of summarising past research topics and interests of the scientific community as well as reflecting past problems and questions. The third outcome is that summarising research trends in a given topic such as pollinators helps pinpointing research gaps and highlight areas in which we have already collected large amounts of data and knowledge. This will help guide future research and make better use of economic resources.

Scope of the study:

Sample: Bee pollinators research articles published in the journal Apidologie from the last 25 years
Phenomenon of interest: Number of articles in each research topic category and year published
Design: Categories used to classify the articles
Evaluation: N/A
Research type: systematic review (qualitative and quantitative method)

Search string (final):

Web of Science: ALL FIELDS: ((pollinat* AND bee*)) Refined by: SOURCE TITLES: (APIDOLOGIE) AND DOCUMENT TYPES: (ARTICLE)

Scopus: (*pollinat** AND *bee**) AND (EXACTSRCTITLE, *"Apidologie"*) AND (DOCTYPE, *"ar"*)

Inclusion criteria for the studies:

All research articles on bee pollinators of all bee ages, including all bee development stages, their hierarchy in a hive, both sexes and both solitary and social bees will be included. Only articles published in the journal Apidologie during approximately the last 25 years will be included. Reviews and meta-analysis publications will be excluded. Below the topic categories included in this study are listed with examples belonging to each category to more easily classify an article's main research topic.

Categories of research:

- **Habitat loss and homogenization**
    - Land-use intensification
    - Agricultural intensification
    - Urbanization

    - Destruction or fragmentation of habitats
    - Decline of abundance and diversity of polliniferous plants and nectar sources, i.e food sources
- **Pesticides**
    - Lethal doses
    - Sublethal doses:
        - Difficulty or unable to collect food (locomotive functions impaired)
        - Influence of memory affecting recognition of flowers and/or where to find them (no pollination)
        - Influence of memory affecting finding their way back to the hive
        - Easier prey
        - Developmental problems
- **Parasites and pathogens and bee microbiome**
    - Affects bee health
    - Influence on production of honey and wax
    - Influence on carrying out pollination
- **Invasive species**
    - Death
    - Competition for food sources
    - Spread of parasites and pathogens
- **Climate change/ climatological**
    - extreme heat, fire and drought
    - hydrological (flooding)
    - meteorological (hurricanes)
    - geophysical (volcanic activity, tsunamis).
- **Mixed categories**
- **Others**
    - Other topics not included in the other main five categories

Protocol for data collection from the full text

The year of publication for each article will be extracted from the title page. Every paper will be assigned to a given category. Information about what categories are covered in the paper will be extracted from the title, abstract, introduction, and aims. After article classification, all articles in every category will be counted and the popularity of each topic in a given year will be presented.

Special cases:

- If articles only fit into one category this will be visualised together with a year of publication in a bar chart.
- If articles fit into two categories it will be visualised in a matrix
- If articles fit into 3 or more categories they will either:
    - Be visualized using a modified color matrix
    - Be visualized in a matrix but counting each article one time per category it is put into.
    - Be put into one category called “mixed categories”

The plan of the study will be modified depending on the outcomes and unexpected situations.

Temporal research popularity in bee pollinators during the last 25 years

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Summary

The abundance and diversity of pollinators have been declining in recent years at an alarming rate, driven by factors such as intensified agricultural practices and climate change. Bee pollinators carry out important ecosystem services and continued losses would cause a threat to human welfare. Despite this, research is lacking in several areas, and directing scientific efforts to find solutions is thus of great importance. Therefore, our aim was to conduct a systematic review of the temporal research trends of bee pollinators from the last 25 years in the bee-dedicated journal Apidologie. We defined research categories and counted the number and type of categories as well as the total number of papers published every odd year from 1995. Our results show a temporal increase in pollinator research with studies focusing on the taxonomy and bee characteristics and social aspects, including nesting biology as the most researched topics in the field included in the journal. The persistent frequency of studies on invasive species was also observed. Our results demonstrate that current research partly is meeting the contemporary need but gaps that require more attention remain. Our review outlines the potential areas of increased research to help identify factors of bee pollinator decline.

Introduction

Anthropogenic impact on the loss of biodiversity has raised many concerns throughout years of debate and is caused by factors such as land-use change, climate change, and invasive species that are tied in the form of economic growth [1]. The loss of biodiversity through land-use change and altered climate poses a major threat to ecosystem functions [2]. The case of insect diversity loss is especially alarming for groups of aquatic insects, Lepidoptera, Hymenoptera, and dung beetles [3]. Many groups of insects provide important ecosystem services that would be at the risk of disappearing if abundance and diversity become too low [4]. One such ecosystem service is pollination. Estimates of flowering plants that depend on pollinators are around 88% and global crops 35% and the estimated value of these ecosystem services is $230–410 billion dollars per year [5,6]. The continued decline of pollinators would thus impose an immense threat to human food production and become an economic burden on human society if pollination was to be done by hand or artificially [7].

Among the different groups that make up pollinators, the bees, both *Apis* and *Bombus* are the most studied groups since the emerging threat of the honey bee *Apis mellifera* parasite *Varroa destructor* in the US [5]. This mite can cause CCD, colony collapse disorder, and much effort has been used in trying to fight this pest [8] due to the importance of the honey bee as an agricultural pollinator. In the US alone

Apis mellifera pollinates one-third of consumed food [9]. Although bee pollinator decline is a multi-faceted problem, Habel et al. [10] identified agricultural intensification as the main driver with strengthened effects of climate change, causing habitat loss and fragmentation. The increased use of pesticides that kill not only the crop pests but all other beneficial insects and invertebrates also presents a major concern for pollinators [11]. Despite this evidence, Havard et al. [12] found lacking research on the impact of climate change and fragmentation on honey bees. This clearly illustrates that there still is a lack of knowledge on bee pollinator research.

Our aim of this study is to survey the patterns and trends in bee pollinator research. Identifying such information is important for three reasons. Firstly, in order to establish that the research conducted is relevant to the modern questions and problems faced. Secondly, analysing the history of research is useful in terms of summarising past research topics and interests of the scientific community as well as reflecting past problems and questions. Thirdly, the outcome of summarising research trends in a given topic such as pollinator decline helps to pinpoint research gaps and highlight areas in which we have already collected large amounts of data and knowledge. This will help guide future research and make better use of research efforts and economic resources.

Material and Methods

We used Web of Science and Scopus to conduct our literature search on 10th of June 2020, using the following search terms for each database; WoS: TS= (pollinat* AND bee*) AND SO=Apidologie, Scopus: Article title, Abstract, Keywords=(pollinat* AND bee*) AND Source title="Apidologie". Searches were only limited to research articles in English but no restriction on year published. The search generated 260 articles for WoS and 484 articles for Scopus see Figure 1. Articles were de-duplicated in Zotero reference manager version 5.0.87. After merging and manual checks 524 articles remained for abstract screening in Rayyan [13]. We excluded reviews, scientific notes, meta-analyses, and modeling articles found. The creation of five research topics guided our screening due to the many study areas covered by our wide article selection. After abstract screening 439 articles still remained and together with a time constraint and the fact that the oldest published article found was from 1987, we narrowed the screening to the last 25 years, excluding all articles before 1995. This removed only 13 abstracts. We narrowed our method further by only including abstracts from every other year starting with the year 1995 to 2019. This resulted in 234 articles for fulltext screening. Full texts not found automatically by Zotero were complemented by the manual search for the pdf. We also extended our five research topic categories into 16 (see supplementary material) to better suit the broad range of topics covered. We removed 14 articles in total from the full-text screening that did not fit the criteria of the year published, were not written in English, or not being a research article, resulting in a final number of 220 articles. The number and type of articles according to established categories were counted as well as the total number of papers published every odd year from 1995. The diagram was created using Excel.

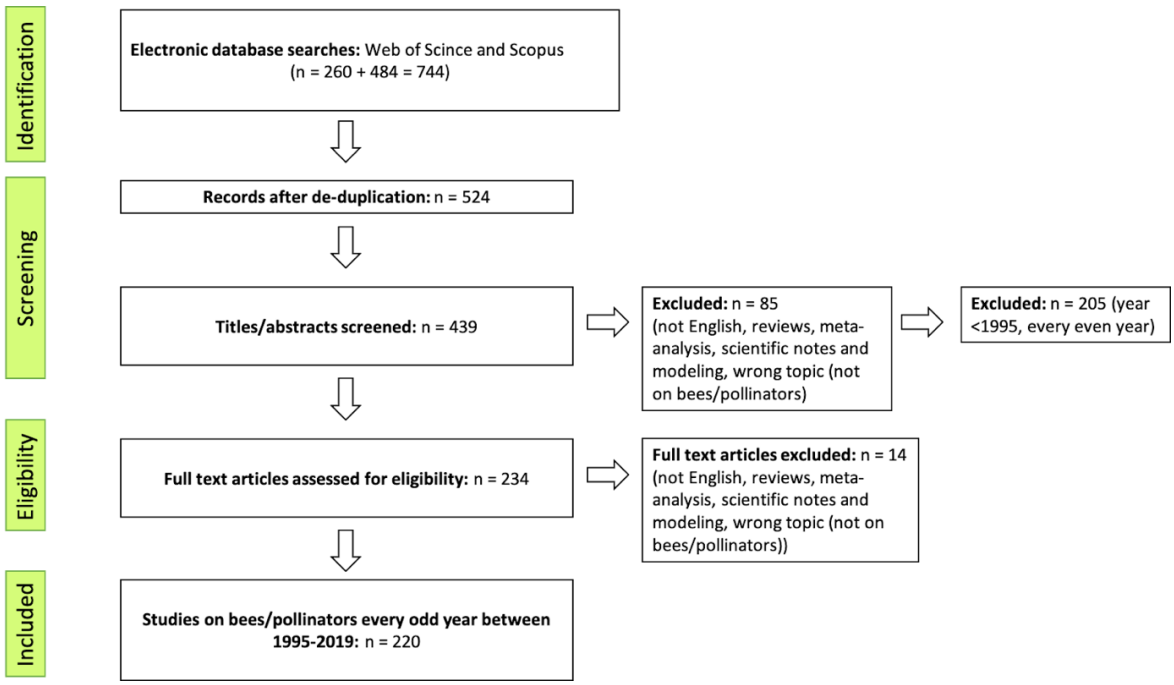

Figure 1. Prisma diagram of our systematic workflow. We added an additional exclusion step when screening abstracts due to the high number of articles remaining and time constraint.

Results

As far as the first investigation is concerned, the results show an increasing trend of research on bee pollinators published in the chosen journal between 1995 and 2019 as can be seen in Figure 2. During the period 1995 to 2009, the total number of articles fluctuated but, from 2011 they started to rise with only a slight decrease in 2019. In Figure 2, we can also observe that in most cases, the popularity of the individual subject areas rose along with the total number of articles during the last 25 years. It also had some fluctuations between 1995 and 2009, then went up until 2019 when it saw a marginal decrease.

The results reveal that the most popular topic of research during these years was taxonomy and bee characteristics, which peaked in 2015. The research topic connected with habitat saw a peak in 2015 but was short-lived, while a gradual growth of interest in the nutrition and social aspects including nesting biology remains prevalent since their inception. The number of foraging studies also became more frequent and stabilised in 2003. Conversely, the rarest topic studied was a combination of foraging and invasive species and remained unchanged through the considered years. A growing field of interest seems to be genetic studies that in 2019 make out a big portion of the research topics published. Furthermore, there are only a few studies on rearing research that had its peak in 2017. The network studies appeared and fluctuated between 2005 and 2019 and they constitute merely a small fraction of all studies in those years.

The research topics published on climate change and abiotic conditions, pesticides and toxicology, and parasites, pathogens, and bee microbiome all fluctuated during the considered years, although the number of published articles remained stable. The other topics which also had minor fluctuations include pollination, enemies, and bee development. There was also an insignificant number of articles classified as others.

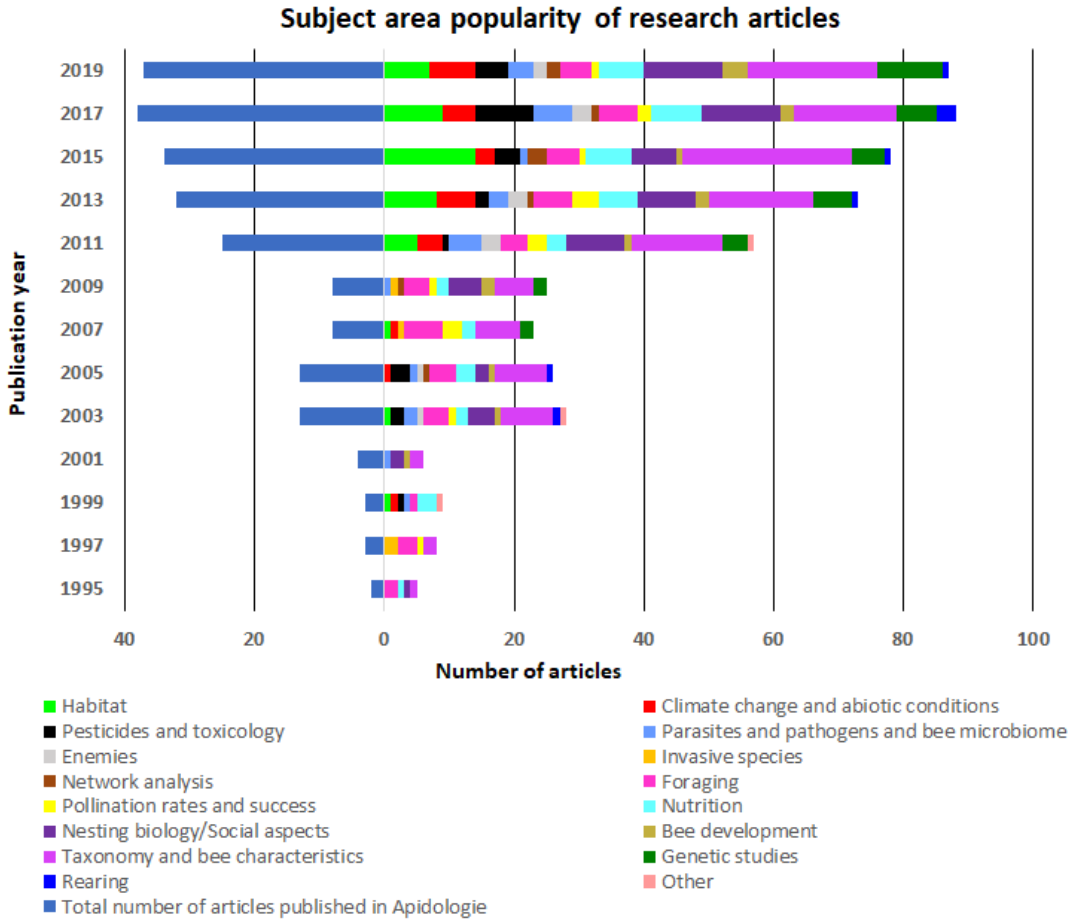

Figure 2. Profile of the particular subject areas of the articles published in Apidologie Journal during the last 25 years and the total number of these articles published in a particular year. The number of the individual subject areas of the articles is shown on the right side of the diagram and the total number of these articles in the respective years is shown on the left side of the diagram. The articles published every second year starting from 1995 were investigated. At least one topic was assigned to every article. Due to the fact every article could be assigned more than one topic, it is counted once in every topic covered. Thus, the total number of articles published in the respective year can be lower.

Discussion

The conservation of bee pollinators is essential not only for maintaining their diversity but also for ecosystem services and consequently human life and economy [14,15]. Partly, as a result of technological advances and lifestyle change, we face an imbalance in the natural environment which can cause long-lasting environmental changes. These changes can trigger the decline of animal abundance, including bees [15]. There is a wide range of threats to bee pollinators such as urbanisation, agricultural intensification, pesticides, climate changes, and invasive species [15–18]. Therefore, it is crucial to fully explore the dangers of bee welfare. We conducted a systematic review to contribute with updated information about research gaps and highlight well-studied areas in the field of bee pollination. We have investigated two questions. First, the total number of the articles on bee pollinators published in Apidologie Journal during the last 25 years within every odd year. The second question concerns the popularity of the particular subject categories of the articles published in Apidologie Journal during the last 25 years. The total number of articles started to rise from 2011, possibly triggered by the increase of public awareness of the existing dangers to bees [19]. The higher frequency of articles could also be the result of an increasing interest in beekeeping. For instance, the beekeeping business in London commanded a higher number of beekeepers which went up by 200% between 2008 and 2013 [20].

The results indicate that the most popular topic of studies during the last 25 years was connected with taxonomy and bee characteristics, mainly the aspects of bee physiology as well as bee specialization and conservation. It is understandable that this topic has a primary focus in science, since a better understanding of the bee metabolism and special behaviour, leads to a better comprehension of their response to different factors. This category remains strongly associated with any other established category due to its significance and the aid of several databases containing taxonomic and genetic information [21]. The next interesting outcome of the results was the high popularity of the topic about social aspects including nesting biology. These topics are important, especially in the case of social behaviour cognition which can largely influence bee survival e.g. during the winter, foraging, and interaction with enemies [22]. As of recently, both of these fields are well studied according to our analysis, the topic is however not saturated [23].

Foraging and nutrition studies are essential for understanding bee survival. The habitat degradation and monocrop economy which limit food sources can cause bee starvation and weakness and it contributed to the bee colony losses during the winter 2007-2008 and 2013-2014 in the USA. Looking at our results, the foraging and nutrition topic popularity in research grew and is maintained in compliance with the needs of the current circumstances. However, it is still difficult to explicitly point out the reasons for bee losses. Scientists stress the lack of epidemiological details included in the loss report but conclude that one main cause of the losses was due to lowered resistance to infections [22,24]. Parasites and pathogens of bees also require continued investigation, especially in times of rapid climate change and human-assisted spread which causes new geographical distributions of harmful enemies and diseases to bees lacking defensive strategies or developed immune systems [25]. According to Brown and Paxton [26] invasive species and parasites pose the second biggest threat to bee populations after habitat loss. This is interesting as climate change and fragmentation have been reported as the main areas lacking research [12]. Our results also suggest an increased need for all these categories of research in the future.

The popularity of genetic studies started to grow in 2011 and rose sharply until 2019. The rise probably owes to the ever-growing advancement and access to technology such as increasingly efficient molecular methods. The complementation of genetic databases could also be meaningful [21,27–29].

The farmers and cultivators all over the world used approximately 5.2 billion pounds of pesticides in 2006 and 2007. The interest of scientists in the topic of pesticides arose only 4 years later. This delay could be a result of the fact that the bee research in this field was carried out then in long-term experiments or the methods of these studies were not optimized yet or there were problems with the studied colonies maintenance. The other reason could be that the effect of bee decline was not visible until much later. As the literature reports, acute poisoning by these chemicals is frequently detected when vast quantities of bees are dead at the hive entrance. And the sublethal effects are more difficult to diagnose. So this field of studies needs faster and more sensitive methods of sensing the bee poisoning caused by pesticides [30].

Although we observe the increasing interest in beekeeping, there is low popularity of rearing research [20]. Our analysis shows that there was a slight growth in this topic frequency 3 years ago but it's probably an insufficient contribution in beekeeping method development and care of bee welfare. As the recapitulated, the subject of queen rearing is too large to present all of its aspects in one paper, let alone the subject of rearing in general [31].

Our systematic review of all articles published during the last 25 years but from only odd years from Apidologie is only a smaller representation of the field. And while bee knowledge is progressively broadened and complemented, there are still important topic areas such as rearing, invasive species, habitat loss, climate change, and network analyses which are far from fully understood. New research

articles in these fields are sought after and research efforts and monetary incentives should aim at expanding and combining these fields to increase our understanding of bee pollinator decline.

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29. Ding G, Zhang S, Huang J, Naeem M, An J, ong. 2019 Colour patterns, distribution and food plants of the Asian bumblebee Bombus bicoloratus (Hymenoptera: Apidae). *APIDOLOGIE* **50**, 340–352.
30. Devillers J, editor. 2014 *In silico bees*. Boca Raton, Florida: CRC Press/Taylor & Francis Group.
31. Büchler R *et al.* 2013 Standard methods for rearing and selection of Apis mellifera queens. *J. Apic. Res.* **52**, 1–30. (doi:10.3896/IBRA.1.52.1.07)

Reviewer: Aneta Arct

This study aimed to perform a systematic review to identify the patterns and trends in bee pollinator research. Systematic reviews are a lot of work, but they are also a valuable part of scientific literature. They allow us to get a big picture of what is known in a field. This study showed only temporal increase in pollinator research with studies focusing on the taxonomy and bee characteristics and social aspects. I am not convinced that this study provide a more complete picture of research knowledge. I have several comments related to the text (see below).

Title: The title are some of the most important parts of a piece. This should be succinct, reflect the nature of the dissertation and contain enough information to attract relevant readers. The surprise captures our attention, and then interest holds it. Firs impression are very important, but my first feeling after reading the title completely discouraged me from reading further.

Summary: The short summary tell me almost everything what I can expect to find in the MS, including some background, scope, how the data were collected, the key findings and conclusion. However, the most important part – aim of the study (line 19-22), it remains unclear. Please to make a clear statement of the importance and purpose of your study, stressing its novelty.
Line 19: Despite this, research is lacking in several areas, and directing scientific efforts to find solutions is thus of great importance – Please convince the reader that we shouldn't be (completely) satisfied with the existing literature on the topic and that your research will address some important limitation or deficiency of existing literature.

Introduction: Too much irrelevant information, introduction should be focused more on the questions or issues that outline the background of the study and establish, using the present tense, the context, relevance, or nature of the problem.
Line 33-55 -it has absolutely nothing to do with the aim of the work.
Line 60-67 - this should precede the sentence with the aim of the study.
The last paragraphs of the 'Introduction' section should include the solution in which you will describe the information you generated, and related data.

Material and Methods: Methods are brief and relatively easy, I didn't find information why the search was limited to only one scientific journal "Apidologie" This point alone, I feel is enough to reject this manuscript as it stands.
Line 78: please explain what research topics
Results: Results described chaotically.
Line 98-99 as can be seen in Figure 2 replace (Fig.2)
Discussion: 129-141 this part is more suited to the introduction,
Line 137-141 at this point I finally found the aims of study!!!!.
I encourage Authors comparing these results to other systematic review, not necessarily performed on pollinator.

Reviewer: Sayantani Basak

Dear authors, the study was an excellent comprehensive and extensive work given the time crunch. I would highlight some minor changes in the text which will help to improve the manuscript further.

General comments

1. The standard format for affiliation is institute, faculty and then university. In case if you want to mention street number, it's better to avoid Polish initials of 'ul'.
2. It would be better to explain in the methodology how you classified five research topics into 16 categories. Is it based on the keywords, own observation or reference to previous studies? This is more important when you make a statement like 'to better suit the broad range of topics covered'. It is good to mention here about the repetitions of some studies into multiple categories than just in the caption of figure 2.

Specific comments

Line 24: from 1995 till when? (even if you mention last 25 years, it is good to state the limit).
Line 28: You mention about gaps that require attention, but it is not preceded by any sentence which says what are the gaps.
Line 48: CCD as an acronym should be within brackets.
Line 53-55: Not very clear.
Line 57: This line is misleading- 'This clearly illustrates that there still is a lack of knowledge on bee pollinator research.' For me, this stands for general bee studies which is actually not. It means environmental factors such as climate change and habitat fragmentation are not well studied. Maybe rewrite as "certain sectors/aspects on bee pollinator research."
Line 71: You did not introduce the acronym 'WoS' before when you mentioned Web of Science.
Line 77: omit 'found'.
Line 77-79: Sentence is confusing.
Line 83: Correct 'full0text'.
Line 84-85: Not required 'Full texts not found automatically by Zotero were complemented by the manual search for the pdf.'
Line 86: In figure 1, check the spelling of 'Web of Science'.
Line 87: Rewrite to PRISMA
Line 89: What is it 16? Maybe rewrite as 'We also extended our five research topics into 16 categories.'
Line 90: Replace 'from' to 'after'.
Line 94: For me this sentence is not always required- 'The diagram was created using Excel.'
Line 97: Rephrase this sentence 'As far as the first investigation is concerned'. It may not be the best statement to start.
Line 108: Inception since when? Maybe stop at prevalent as the inception maybe even before 1995.
Line 109: frequent and stabilise? Could stabilise be replaced with increase?
Line 109: Change rarest to least.
Line 151: Change 'any' to 'many'.
Line 177-178: If using numbers (e.g. 5.2 billion), it is important to back it with citations.
Line 183: By 'these chemicals' did you mean pesticides as you previously did not mention any chemical. To make the transition smooth, you can start the sentence in line 178 as 'chemical pesticides.'
Line 184: Avoid starting a sentence with 'And'.
Line 183: Change 'chemicals is' to 'chemicals are'
Line 188: 'frequency' not needed.
Line 192-193: 'Our systematic review of all articles published during the last 25 years but from only odd years from Apidologie is only a smaller representation of the field' This line is contradictory when you mention 'all'. You can instead rewrite as 'Our systematic review of articles published in every

alternate (or odd) years during the last 25 years in Apidologie is only a smaller representation of the field'.
Line 193: Not the best approach to begin a sentence with 'and', specially at the very end of the discussion.
Line 194: Remove 'topic'.

Reviewer: Ewa Szlachcic

The study is a systematic review of papers from the chosen journal dedicated to bees in order to detect the temporal research trends of bee pollinators observed in the last 25 years. I think that this research is informative and valuable not only to specialists in the pollination field, but also to the general readers and should be published in the Journal. The title, summary and introduction section provide interesting information and they are understandable for non-specialists. It can lead to popularization of science and to raising human awareness about the existing threats to the bees. Moreover, I think that title and abstract properly describe contents of the paper. In my opinion reviewed study presents an important synthesis of trends in past research in pollination field. Furthermore, it shows where the gaps are and what should be done in the future which is essential taking into consideration that pollinators provide important ecosystem services. As far as I know it is the first study in the pollination field which considers such approach. The aim is clarified and all sections of the paper are consistent.

The length and structure of the text is appropriate. However, it would be more useful to include keywords and change the order of words in affiliation, starting from the Institute. The colour used in Figure 2 is necessary for a better comprehension of its content. The title above Figure 2 is unnecessary or could be moved to the description (line 121). Methods are written in detail, but they lack the description of how papers were classified into each category. This information is added under the Figure 2 but in my opinion should be also included in the methods section. Moreover, Materials and Methods part should provide information about the program used to visualize data presented in Figure 2. In order to avoid ambiguity I would mention in line 89 that research topic categories are also called subject areas. Additionally, in line 94 I would suggest to put in brackets the full information about the Excel program.

Temporal research popularity in bee pollinators during the last 25 years - do the contemporary problems and scientific effort match?

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Summary

The abundance and diversity of pollinators have been declining in recent years at an alarming rate, driven by factors such as intensified agricultural practices and climate change. Bee pollinators carry out important ecosystem services and continued losses would cause a threat to human welfare. Despite this threat, research is lacking and directing scientific efforts to find solutions is thus of great importance. Therefore, our aim was to conduct a systematic review of the temporal research trends of bee pollinators from the last 25 years in the bee-dedicated journal Apidologie to aid the direction of future research. We counted the number and type of defined research categories as well as the total number of papers published every odd year between 1995-2019. Results show a temporal increase in pollinator research with studies focusing on taxonomy and bee characteristics and social aspects, including nesting biology, as the most researched topics published in the journal. The persistent frequency of studies on invasive species was also observed. Our results demonstrate that current research partly is meeting the contemporary need, but gaps that require more attention remain. Our review outlines the potential areas of increased research to help identify factors of bee pollinator decline.

Keywords: bee pollinators, bees, popularity, gaps, research topics

Introduction

Anthropogenic impact on the loss of biodiversity has raised many concerns throughout years of debate and is caused by factors such as land-use change, climate change, and invasive species that are tied in the form of economic growth [1]. The loss of biodiversity through land-use change and altered climate poses a major threat to ecosystem functions [2]. The case of insect diversity loss is especially alarming for groups of aquatic insects, Lepidoptera, Hymenoptera, and dung beetles [3]. Many groups of insects provide important ecosystem services that would be at the risk of disappearing if abundance and diversity become too low [4]. One such ecosystem service is pollination. Estimates of flowering plants that depend on pollinators are around 88% and global crops 35% and the estimated value of these ecosystem services is $230–410 billion dollars per year [5,6]. The continued decline of pollinators would thus impose an immense threat to human food production and become an economic burden on human society if pollination was to be done by hand or artificially [7].

Among the different groups that make up pollinators, the bees, both *Apis* and *Bombus* are the most studied groups since the emerging threat of the honey bee *Apis mellifera* parasite *Varroa destructor* in the US [5]. This mite can cause colony collapse disorder (CCD), and much effort has been used in trying to fight this pest [8] due to the importance of the honey bee as an agricultural pollinator. In the US alone *Apis mellifera* pollinates one-third of consumed food [9]. Although bee pollinator decline is a multi-faceted problem, Habel et al. [10] identified agricultural intensification as the main driver with strengthened effects of climate change, causing habitat loss and fragmentation. The increased use of pesticides that kill not only the crop pests but all other beneficial insects and invertebrates also presents a major concern for pollinators [11] as they feed on the nectar and pollen containing either sublethal or lethal doses of the systemic pesticides [12]. Despite this evidence, Havard et al. [13] found lacking research on the impact of climate change and fragmentation on honey bees. This clearly illustrates that there still is a lack of knowledge and research on how environmental factors and human impact affects bee pollinators.

Our aim of this study is to survey the patterns and trends in bee pollinator research. Identifying such information is important for three reasons. Firstly, in order to establish that the research conducted is relevant to the modern questions and problems faced. Secondly, analysing the history of research is useful in terms of summarising past research topics and interests of the scientific community as well as reflecting past problems and questions. Finally, the outcome of summarising research trends in a given topic such as pollinator decline helps to pinpoint research gaps and highlight areas in which we have already collected large amounts of data and knowledge. This will help guide future research and make better use of research efforts and economic resources. We investigated two questions. First, the total number of the articles on bee pollinators published in Apidologie Journal during the last 25 years within every odd year. The second question concerns the popularity of the particular subject categories of the articles published in Apidologie Journal during the last 25 years within every odd year.

Materials and Methods

We used Web of Science (WoS) and Scopus to conduct our literature search on 10th of June 2020, using the following search terms for each database; WoS: TS= (pollinat* AND bee*) AND SO=Apidologie, Scopus: Article title, Abstract, Keywords=(pollinat* AND bee*) AND Source title="Apidologie". We limited our search to the journal Apidologie since it is dedicated to all research on bees, has been active for many years, and thus had many articles published from various fields. Searches were only limited to research articles in English but no restriction on year published. The search generated 260 articles for WoS and 484 articles for Scopus (see Figure 1.). Articles were de-duplicated in Zotero reference manager (version 5.0.87). After merging and manual checks, 524 articles remained for abstract screening in Rayyan [14]. We excluded reviews, scientific notes, meta-analyses, and modeling articles. We included all abstracts that contained mention of bees of any age, including all bee development stages, their hierarchy in a hive, both sexes, solitary and social bees, wild and domesticated ones. After abstract screening 439 articles still remained and together with a time constraint and the fact that the oldest published article found was from 1987, we narrowed the screening to the last 25 years, excluding all articles before 1995. This removed only 13 abstracts. We narrowed our method further by only including abstracts from every other year starting with the year 1995 to 2019. This resulted in 234 articles for full-text screening. Full texts not found automatically by Zotero were complemented by the manual search for the pdf.

Due to the many study areas covered by our wide article selection, we created five categories of research topics: habitat loss and homogenization, pesticides and toxicity, parasites and pathogens and bee microbiome, invasive species, climate change/climatological, rearing and other for our full-text screening. Every paper would be assigned at least one category. Information about which categories to

put the articles in was extracted from the full text by primarily reading the title, abstract, introduction to find the aims and target of research.

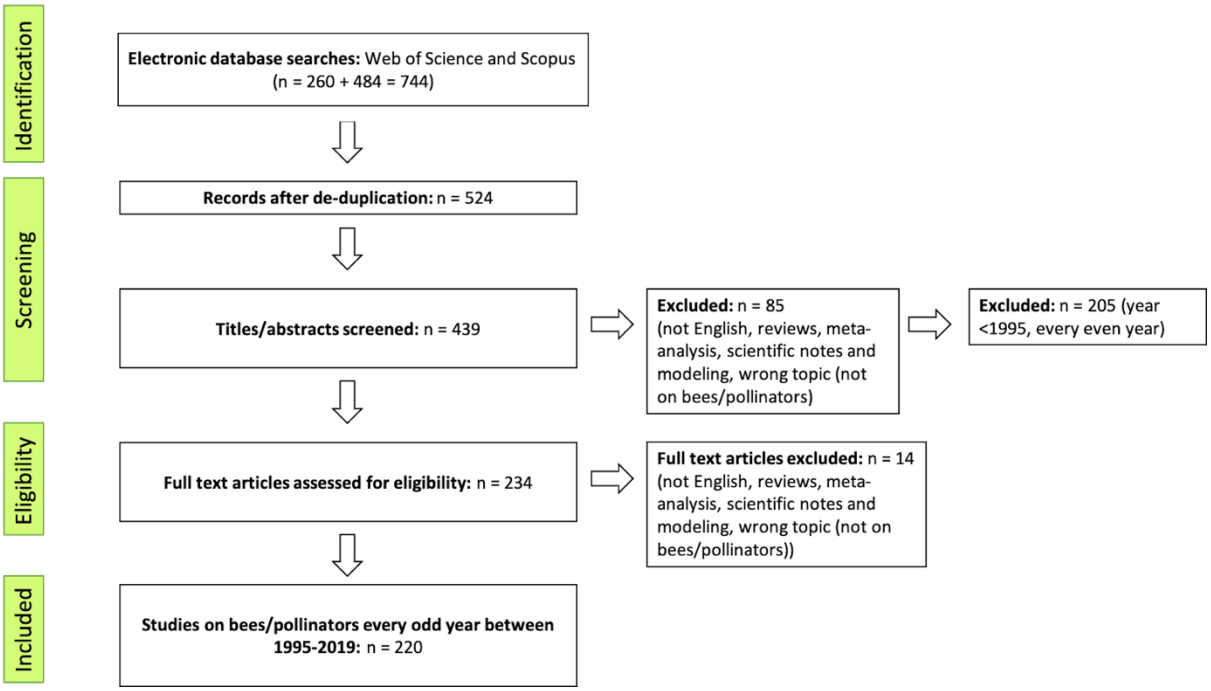

Figure 1. PRISMA diagram of our systematic workflow. We added an exclusion step when screening abstracts due to the high number of articles remaining and time constraint.

During the early stage of full-text screening, we redefined our five research topic categories (also called subject areas) and extended them into 16 categories (see supplementary material) to better suit the broad range of topics covered. We removed 14 articles in total after the full-text screening that did not fit the criteria of the year published, were not written in English, or not being a research article, resulting in a final number of 220 articles. The number and type of articles according to established categories were counted as well as the total number of papers published every odd year from 1995 to 2019. Owing to the fact that one article could be placed in more than one category, it was counted once for each category which means that the total number of articles published could be lower in the subsequent visualization. The result diagram was created using Microsoft Excel 365.

Results

The results show an increasing trend of research on bee pollinators published in the chosen journal between 1995 and 2019 as can be seen in Figure 2. During the period 1995 to 2009, the total number of articles fluctuated but, from 2011 they started to rise with only a slight decrease in 2019. In Figure 2, we can also observe that in most cases, the popularity of the individual subject areas rose along with the total number of articles during the last 25 years. It also had some fluctuations between 1995 and 2009, then went up until 2019 when it saw a marginal decrease.

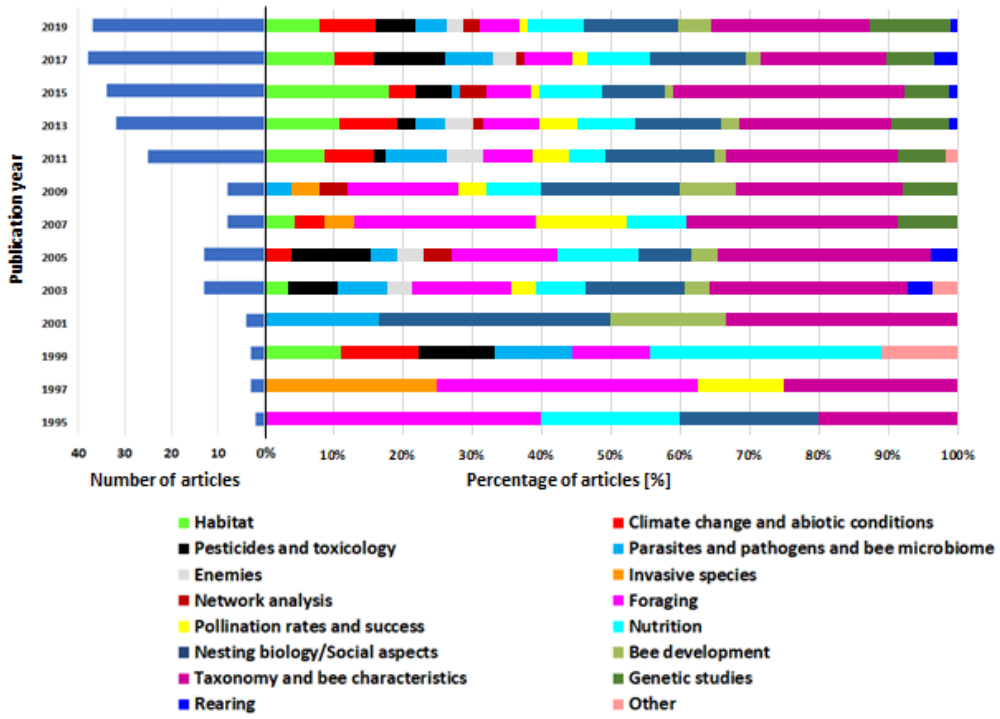

Figure 2. Profile of the particular subject areas of the articles published in Apidologie Journal during the last 25 years and the total number of these articles published in a particular year. The percentage of the number of the individual subject areas of the articles is shown on the right side of the diagram and the total number of these articles in the respective years is shown on the left side of the diagram. The articles published every second year starting from 1995 were investigated. At least one topic was assigned to every article.

The results reveal that the most popular topic of research during these years was taxonomy and bee characteristics, which peaked in 2015. The research topic connected with habitat saw a peak in 2015 but was short-lived, while a gradual growth of interest in the nutrition and social aspects including nesting biology remains prevalent. The number of foraging studies also became more frequent in 2003. Conversely, the least studied topic was a combination of the categories foraging and invasive species which remained unchanged through the considered years. A growing field of interest seems to be genetic studies that in 2019 make out a big portion of the research topics published. Furthermore, there are only a few studies on rearing research that had its peak in 2017. The network studies appeared and fluctuated between 2005 and 2019 and they constitute merely a small fraction of all studies in those years. The research topics published on climate change and abiotic conditions, pesticides and toxicology, and parasites, pathogens, and bee microbiome all fluctuated during the considered years, although the number of published articles remained stable. The other topics which also had minor fluctuations include pollination, enemies, and bee development. There was also an insignificant number of articles classified as others.

Discussion

The conservation of bee pollinators is essential not only for maintaining their diversity but also for ecosystem services and consequently human life and economy [15,16]. Partly, as a result of technological advances and lifestyle change, we face an imbalance in the natural environment which can cause long-lasting environmental changes. These changes can trigger the decline of animal abundance, including bees [16]. There is a wide range of threats to bee pollinators such as urbanisation, agricultural intensification, pesticides, climate changes, and invasive species [16–19]. Therefore, it is crucial to fully explore the dangers of bee welfare. We conducted a systematic review to contribute with updated information about research gaps and highlight well-studied areas in the field of bee pollination. We have investigated two questions. First, the total number of the articles on bee pollinators published

in Apidologie Journal during the last 25 years within every odd year. The second question concerns the popularity of the particular subject categories of the articles published in Apidologie Journal during the last 25 years within every odd year. The total number of articles started to rise from 2011, possibly triggered by the increase of public awareness of the existing dangers to bees [20]. The higher frequency of articles could also be the result of an increasing interest in beekeeping. For instance, the beekeeping business in London commanded a higher number of beekeepers which went up by 200% between 2008 and 2013 [21].

The results indicate that the most popular topic of studies during the last 25 years was connected with taxonomy and bee characteristics, mainly the aspects of bee physiology as well as bee specialization and conservation. It is understandable that this topic has a primary focus in science, since a better understanding of the bee metabolism and special behaviour, leads to a better comprehension of their response to different factors. This category remains strongly associated with many other established categories due to its significance and the aid of several databases containing taxonomic and genetic information [22]. The next interesting outcome of the results was the high popularity of the topic about social aspects including nesting biology. These topics are important, especially in the case of social behaviour cognition which can largely influence bee survival e.g. during the winter, foraging, and interaction with enemies [23]. As of recently, both of these fields are well studied according to our analysis, the topic is however not saturated [24].

Foraging and nutrition studies are essential for understanding bee survival. The habitat degradation and monocrop economy which limit food sources can cause bee starvation and weakness and it contributed to the bee colony losses during the winter 2007-2008 and 2013-2014 in the USA. Looking at our results, the foraging and nutrition topic popularity in research grew and is maintained in compliance with the needs of the current circumstances. However, it is still difficult to explicitly point out the reasons for bee losses. Scientists stress the lack of epidemiological details included in the loss report but conclude that one main cause of the losses was due to lowered resistance to infections [23,25]. Parasites and pathogens of bees also require continued investigation, especially in times of rapid climate change and human-assisted spread which causes new geographical distributions of harmful enemies and diseases to bees lacking defensive strategies or developed immune systems [26]. According to Brown and Paxton [27], invasive species and parasites pose the second biggest threat to bee populations after habitat loss. This is interesting as climate change and fragmentation have been reported as the main areas lacking research [13]. Our results also suggest an increased need for all these categories of research in the future.

The popularity of genetic studies started to grow in 2011 and rose sharply until 2019. The rise probably owes to the ever-growing advancement and access to technology such as increasingly efficient molecular methods. The complementation of genetic databases could also be meaningful [22,28–30].

The farmers and cultivators all over the world used approximately 5.2 billion pounds of pesticides in 2006 and 2007. The interest of scientists in the topic of pesticides arose only 4 years later [31]. This delay could be a result of the fact that the bee research in this field was carried out then in long-term experiments or the methods of these studies were not optimized yet or there were problems with the studied colonies maintenance. The other reason could be that the effect of bee decline was not visible until much later. As the literature reports, acute poisoning by these pesticides are frequently detected when vast quantities of bees are dead at the hive entrance. However, sublethal effects are more difficult to diagnose. So this field of studies needs faster and more sensitive methods of sensing the bee poisoning caused by pesticides [31].

Although we observe the increasing interest in beekeeping, there is low popularity of rearing research [21]. Our analysis shows that there was a slight growth in this topic 3 years ago but it’s probably an

insufficient contribution in beekeeping method development and care of bee welfare. As the recapitulated, the subject of queen rearing is too large to present all of its aspects in one paper, let alone the subject of rearing in general [32].

Our systematic review of articles published in every alternate year during the last 25 years in Apidologie is only a smaller representation of the field of pollinator research trends and is thus a clear limitation to our study. Ideally, several journals would be compared as well as every year to get a more holistic picture and enable the possibility of meta-analysis which is not possible in our current findings and scope.

While bee pollinator knowledge is progressively broadened and complemented, there are still important areas such as rearing, invasive species, habitat loss, climate change, and network analyses which are far from fully understood. New research articles in these fields are sought after to match the current problems and research efforts and monetary incentives should aim at expanding and combining these fields to increase our understanding of bee pollinator decline.

Acknowledgements

We would like to thank our professor and colleagues for useful input to improve this manuscript.

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32. Büchler R *et al.* 2013 Standard methods for rearing and selection of Apis mellifera queens. *J. Apic. Res.* **52**, 1–30. (doi:10.3896/IBRA.1.52.1.07)

Supplementary material

The established criteria (16 research topic categories)

Categories of research:

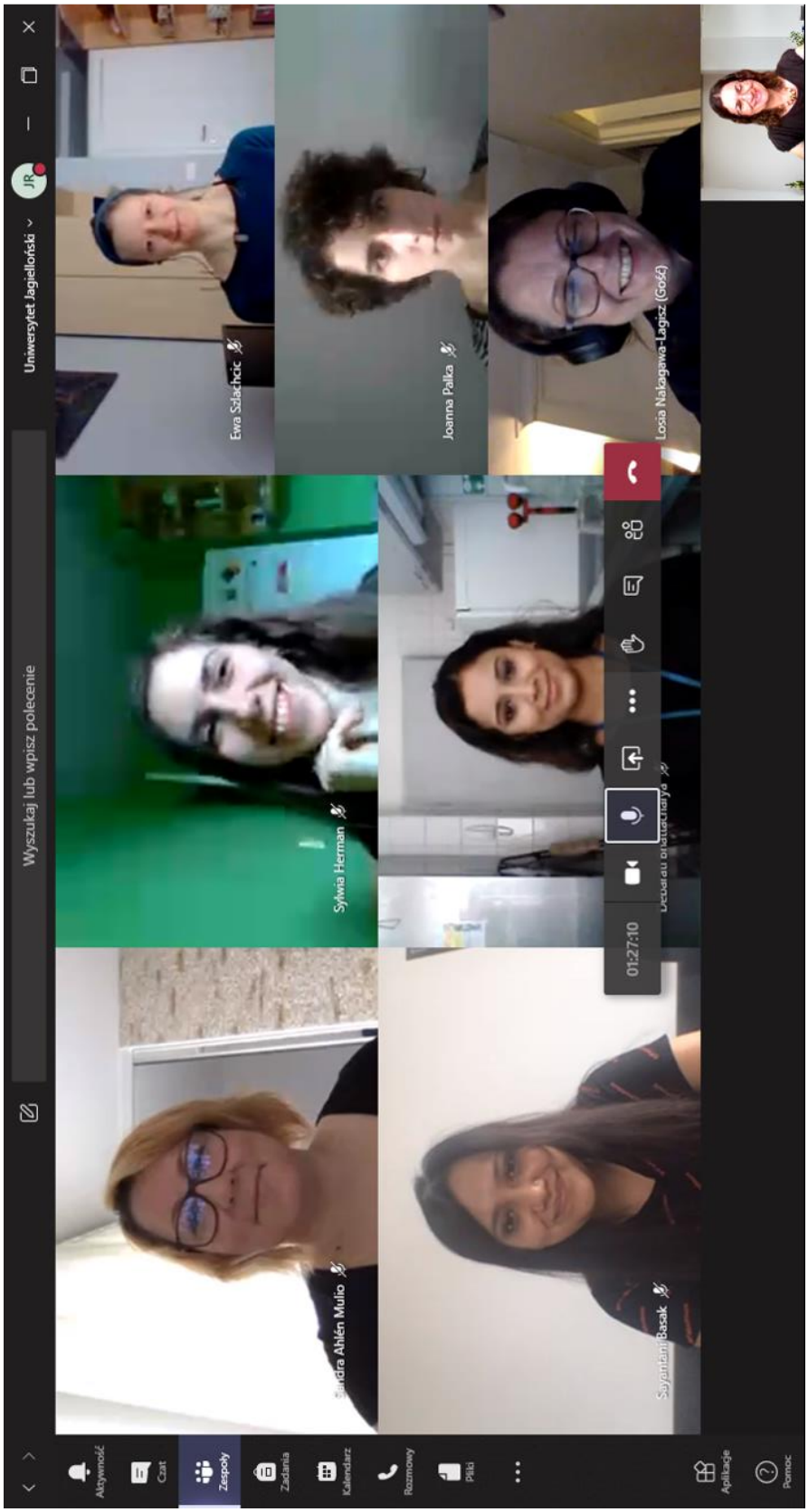
- **Habitat**
  - Land-use intensification
  - Loss and homogenization
  - Agricultural intensification
  - Urbanization
  - Air pollutants
  - Destruction or fragmentation of habitats

    - Decline of abundance and diversity of polliniferous plants and nectar sources, i.e food sources
    - Availability of food sources
    - Area of bee occurrence and distribution
    - Bee behaviour special for habitat
    - Improvements of methods in studies about bee habitat
- **Pesticides and toxicology**
    - Lethal doses
    - Sublethal doses:
        - Difficulty or unable to collect food (locomotive functions impaired)
        - Influence of memory affecting recognition of flowers and/or where to find them (no pollination)
        - Influence of memory affecting finding their way back to the hive
        - Easier prey
        - Developmental problems
    - Bee behaviour associated with pesticide or toxic substance influence
    - Mycotoxins
    - Plant toxicity
    - Resistance/tolerance
    - Improvements of methods in studies about pesticide or toxic substance influence on bees
- **Parasites and pathogens and bee microbiome**
    - Affects bee health
    - Influence on production of honey and wax
    - Influence on carrying out pollination
    - Bee behavioural response to parasites/pathogens/microbiome
    - Improvements of methods in studies about parasite or pathogen influence on bees and in studies about bee microbiome
    - Antibiotics and treatment methods
- **Invasive species**
    - Death
    - Competition for food sources
    - Spread of parasites and pathogens
    - Differences in colony strength
    - Bee behaviour associated with response to invasive species
    - Improvements of methods in studies about invasive species influence on bees
- **Climate change/ climatological/ abiotic conditions**
    - season
    - extreme heat, fire and drought
    - hydrological (flooding)
    - meteorological (hurricanes)
    - geophysical (volcanic activity, tsunamis).
    - Bee behaviour associated with climate changes/ abiotic conditions
    - Improvements of methods in studies about climate change or abiotic condition influence on bees
- **Genetic studies**
    - Phylogeny
    - Improvements of methods in bee genetic studies
- **Nesting biology/Social aspects**
    - Architecture

    - Nesting ecology
    - Nesting admeasurement (e.g. colony strength)
    - Colony demography
    - Interactions in the nest and between different species
    - Bee behaviour associated with nest, e.g. dead brood removal
    - Population size
    - Food reserves
    - Overwintering
    - Improvements of methods in bee nesting and social aspects studies
- **Foraging**
    - Preferences
    - Frequency
    - Distance
    - Bee behaviour associated with foraging
    - Improvements of methods in bee foraging studies
    - Competition
- **Nutrition**
    - Pollen studies
    - Nectar studies
    - Bee behaviour associated with nutrition
    - Metabolism
    - Amounts and timing of feeding
    - Improvements of methods in bee nutrition studies
    - Diet breadth
    - Food odours
- **Predation/parasitoids/kleptoparasites**
    - Bee behaviour associated with predator defence
    - Improvements of methods in studies about predator, parasitoid or kleptoparasite influence on bees
- **Pollination rates and success**
    - Bee behaviour associated with pollination
    - Pollen measurements
    - Greenhouse
    - Improvements of pollination methods
- **Rearing**
    - Beekeeping practice
    - Rearing conditions
    - in vitro rearing methods
    - Risk assessment and its methods
    - Feeding methods
    - Improvements of methods in bee rearing studies
- **Network analysis**
    - Food webs
    - Interaction between species
    - Alfa-, beta-, gamma diversity
    - Improvements of methods in bee network studies
- **Bee development**
    - Comparison between different bee development stages
    - Significant factor influence on particular bee development stages
- **Taxonomy and bee characteristics**

    - Physiology - bodily functions (e.g. metabolism, wing beat rate etc.)
    - Morphology
    - Diversity of bees and their specialization
    - Bee behaviour associated with particular taxon
    - Improvements of methods in bee taxonomy studies
    - Bee identification
    - Conservation
- **Others**
    - Other topics not included in the other categories

FINAL DAY OF ONLINE COURSE PHOTO



Thanks for a fun and valuable course!