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Review

Space omics research in Europe: Contributions, geographical distribution and ESA member state funding schemes

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SUMMARY

The European research community, via European Space Agency (ESA) spaceflight opportunities, has significantly contributed toward our current understanding of spaceflight biology. Recent molecular biology experiments include "omic" analysis, which provides a holistic and systems level understanding of the mechanisms underlying phenotypic adaptation. Despite vast interest in, and the immense quantity of biological information gained from space omics research, the knowledge of ESA-related space omics works as a collective remains poorly defined due to the recent exponential application of omics approaches in space and the limited search capabilities of pre-existing records. Thus, a review of such contributions is necessary to clarify and promote the development of space omics among ESA and ESA state members. To address this gap, in this review, we i) identified and summarized omics works led by European researchers, ii) geographically described these omics works, and iii) highlighted potential caveats in complex funding scenarios among ESA member states.

INTRODUCTION

The global drive to walk on the surface of the Moon and ultimately Mars, combined with the rise of commercial spaceflight, will lead to an unprecedented number and heterogeneous population of humans entering space for short and long(er) periods of time (Rutter et al., 2020; Schmidt and Goodwin, 2013). Spacefarers will inevitably experience higher exposures to the environmental stressors associated with spaceflight, such as microgravity and ionizing radiation (Furukawa et al., 2020), which induce numerous negative health adaptations including but not limited to: skeletal muscle and bone loss (LeBlanc et al., 1998; Tominari et al., 2019; Vandenburgh et al., 1999), altered immune responses (Crucian et al., 2018; Konstantinova, 1991), and visual impairment (Lee et al., 2018; Zhang and Hargens, 2018). Ultimately, these can impact an astronaut's inflight operations, ambulation upon return to Earth, and overall health (Buckey, 1999). As such, there is an urgent need to unravel the underlying mechanisms regulating such biological (mal)adaptation and to devise effective countermeasures to ensure safe space travel. Further, optimizing other elements of the spaceflight infrastructure, such as limiting pathogen infection, culturing plants and microbes for bioregenerative life support and nutrient sources, and designing specific nutritional interventions (Rutter et al., 2020), is critical to interplanetary exploration and habitation success.

The European Space Agency (ESA) has significantly contributed toward the unraveling of (extra)terrestrial biological phenomena and our current understanding of spaceflight biology, demonstrated by the support of life science experiments performed during spaceflight missions either in real microgravity on orbital platforms or space research analogs (e.g., simulated microgravity), utilizing a variety of model organisms (ESA Erasmus Experiment Archive). More recently, a selection of these molecular biology experiments have performed "omics" analysis (i.e., the comprehensive/global assessment of a particular set of biological molecules (Hasin et al., 2017)), which maximizes the knowledge gained from unique spaceflight experiments (Rutter et al., 2020) and provides a more holistic and systems level understanding of the mechanisms underlying phenotypic adaptation. Unsurprisingly, the current application of omics analysis continues at unabated rates, likely due to technological advances driving down the cost of high-throughput analysis and computational analysis pipelines becoming more widely exploitable (Misra et al., 2018). Indeed, such rapid progress was the driving force for establishing the Space Omics Topical Team (Madrigal

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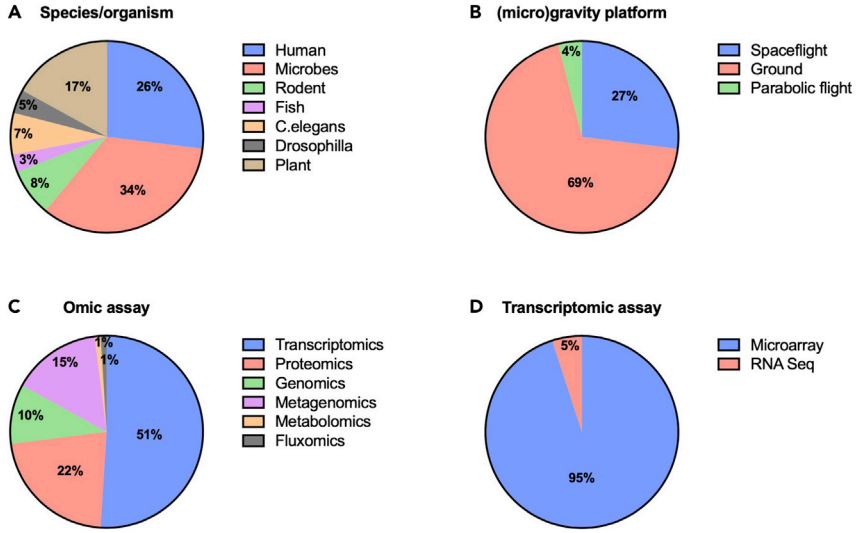

Figure 1. Types of experimental studies co(funded) by ESA that have an omic component
Studies are sorted by species/organism (A), (micro)gravity platform (B), omic assay (C), and transcriptomic assay type (D).

et al., 2020), which aims to support the use of omics research among the ESA space biology community. Despite vast interest in, and the immense quantity of biological information gained from space omics research, the knowledge of ESA-related space omics works as a collective and remains poorly defined as current records (i.e., ESA Erasmus Experiment Archive) cannot be easily leveraged due to the limited search capabilities. However, a better understanding of such contributions is necessary to unify and promote the development of space omics among ESA and ESA state members. This is particularly relevant if European state members want to keep pace with global omics efforts, which has seen a boom in activity since the initiation of the National Aeronautic Space Administration (NASA) GeneLab data repository, which allows the sharing of spaceflight omics data and provides improved visualization and analysis tools free to the public (Ray et al., 2019).

Therefore, the purpose of this review is to provide a historical summary of Europe's contribution to space omics from when records began through to summer 2020. Indeed, such research is continually being conducted, with numerous research articles more recently published in several special issues/collections (e.g (Cahill et al., 2021; Madrigal et al., 2020). As such, our first aim was to identify and summarize peer-reviewed experimental publications performed in spaceflight or ground-based simulations, which include omics analysis and were (co)funded by ESA. Our second aim was to geographically describe ESA/European omics contributions. Our final aim was to highlight possible caveats in the distribution and type of contributions based on differential funding scenarios for research across ESA member states being accountable for the spaceflight and/or ground-based facilities access and payload development only.

ESA-(CO)FUNDED OMICS WORKS

In total, we identified 75 individual relevant studies that were (co)funded by ESA *and* utilized omics analysis (Tables S1–S7), with an additional 20 works identified that did not acknowledge ESA funding, but did involve European input (Table S8, see methodological details). Of note, most of these studies had relevance to spaceflight research, although some of them do lack spaceflight culture conditions and/or reference to a particular spaceflight experiment. Nonetheless, these studies were (co)funded by ESA and had an omics component, and so were deemed to have relevance to the European spaceflight omics community and they were thus included. Out of the 75 ESA-(co)funded studies, 65 were identified via Web of Science (Methodological details) and 10 were manually identified by the current authors (i.e., these studies did not appear in the original Web of Science searches, but were known to the authors to meet the search criteria). This diverse set of studies included data from a vast array of species/models including humans (and human cell lines), rodents, fish, invertebrates (mainly *Drosophila melanogaster* and *Caenorhabditis elegans*), plants (e.g., *Arabidopsis*), and microbes (including bacteria, fungi, and algae) (Figure 1A). While the largest percentage of the research has been done on microbes (34%), this is likely due to the bundling of many different

models (i.e., bacteria *and* fungi *and* algae) under the single "microbes" term. Of all 75 studies, a large percentage (69%) of studies were performed exclusively on the ground (i.e., Earth); although, this does include simulated microgravity conditions, for example, those utilizing the random positioning machine. In contrast, only 27% of studies investigated spaceflight-exposed samples, including stays on the International Space Station (ISS) and the use of biosatellites (e.g., Bion-M1 biosatellite) (Figure 1B). A variety of omic profiling was used across these studies, including genomics, transcriptomics, proteomics, and metagenomics (Figure 1C). By far, the most used omic was transcriptomics (51%), with 95% of transcriptomic profiling based on microarray versus only 5% percent based on RNA sequencing (Figure 1D). The application of RNA sequencing within these ESA-(co)funded studies emerged in publications from 2018 onwards, which likely reflects the now easier access and lower costs associated with RNA sequencing. Somewhat surprisingly, the application of RNA sequencing (i.e., 2018 onwards) is delayed compared to NASA- or JAXA-funded studies, which may be due to the slow recovery of biological spaceflight experiment opportunities in Europe after the discontinuation of some funding programs following the 2008 financial crisis. It is also possible that literature searching-associated caveats could have omitted such studies (see 'limitations'). Nonetheless, it was possible to identify 33 datasets on the Genelab repository that acknowledge ESA contributions and 44 datasets where the Space Omics Topical Team contributed to the generation and/or analysis. Thus, 65 datasets in total—excluding overlaps— accounting for approximately 1/5 of the whole GeneLab repository (Table S9).

Summary of European space omics contributions in microbes

Microbes are among the most studied organisms in space due to their size, cost effectiveness, ease of transport, relatively low maintenance, and the ability to collect fresh samples (i.e., profiling sample swabs taken on the ISS). However, the full potential of metagenomics and metatranscriptomics has only begun to be explored, with most of the studies supported by ESA and other agencies using sequencing technologies on a limited scale, mainly transcriptomics on experimental designs with single or few microbes (Table S1). Unleashing the full potential of sequencing technologies is becoming crucial to characterize microbial communities and to isolate potential pathogens (Venkateswaran et al., 2017)(Table S1).

A number of microbe studies coming from the Belgian research centers have expanded our understanding of transcriptional and translational de-regulation due to spaceflight exposure or simulated conditions of microgravity and radiation. Initial Micro-Ecological Life Support System Alternative (MELiSSA) project flight and ground-based experiments showed that *Rhodospirillum rubrum* senses and reacts to microgravity and ionizing radiation, exhibiting a more pronounced response at the transcriptomic rather than the proteomic level (Mastroleo et al., 2009). Later ground-based experiments on photoheterotrophic acute stress showed that reversible genome amplification and overexpression of enzymes of the ethylmalonyl-CoA pathway are involved in *R. rubrum* stress adaptation, carbon metabolism, and redox balance (De Meur et al., 2018). In the MELiSSA context, an extremely triclosan-responsive cluster of four *muf* (micropollutant-upregulated factor) genes were discovered in *R. rubrum* (Pycke et al., 2010). A study by the same group on *R. rubrum* S1H using microgravity simulators (i.e., rotating wall vessel and random positioning machine) provided evidence of changes in the transcriptome, proteome, and metabolome compared to normal gravity control (Mastroleo et al., 2013). A later study showed metabolic alterations in *R. rubrum* when assimilating volatile fatty acids (generated by fermentative biodegradation of organic waste) upon exposure to photoheterotrophic stress conditions (De Meur et al., 2020). Metabolic changes were also found in *Pseudomonas putida* after culturing in high-shaking speed conditions (non-filament inducing) when compared to low-shaking (filament inducing) (Crabbe et al., 2012).

The MELiSSA project has also investigated the oxygenic and waste recycling potential of *Arthrospira sp*. for production of edible biomass and oxygen for crewed space missions (Sachdeva et al., 2018). Microarray analysis of *Pseudomonas aeruginosa* PAO1 grown in the low-shear modeled microgravity demonstrated the induction of alternative sigma factor, AlgU, and genes under its regulation, when compared to normal gravity control (Crabbe et al., 2010). Several publications largely focused on proteomics (Badri et al., 2015; Deschoenmaeker et al., 2017; Matallana-Surget et al., 2014) have untangled the relation between photosensing and antioxidant systems in ground-based facilities including exposure to gamma radiation by *Arthrospira* or the impact of different nitrogen sources required for optimal plant nutrition in the context of autonomous life-support systems.

Steps have also been taken to improve protocols for interrogation of bacterial proteomes, for example, a lysis protocol that can be used to extract genomic DNA from both Gram-positive and Gram-negative species without interfering with the amplification chemistry has been developed (Leroy et al., 2010; Liu et al., 2018). Furthermore, sample preservation methods compatible with various omics technologies have been developed for *Saccharomyces cerevisiae*, a model system previously used in microgravity experiments on the ISS (van Eijsden et al., 2013).

One way of investigating the adaption of life to extreme conditions has been to study how bacteria adapt to the presence of toxic heavy metals, with the *Cupriavidus metallidurans* strain CH34 considered the model organism of choice (Janssen et al., 2010). Furthermore, how the genetic variation between different strains influences the response of these bacteria has been investigated to characterize metal resistance determinants shared by all *C. metallidurans* strains using whole-genome oligonucleotide DNA microarrays (Van Houdt et al., 2012). Metal resistance determinants were found to be maintained in all strains, with only minor differences observed in the global phenomes (as measured by phenotype microarrays) (Van Houdt et al., 2018). A strain-dependent microgravity response has been found in *S. cerevisiae* with respect to proteome and colony growth (Van Mulders et al., 2011).

Determination of both microbial abundance and diversity on the ISS and in spacecrafts (clean rooms) *en route* to other planets is currently a hotbed of research for space agencies as they may represent a threat to human health and a contamination risk to other planets. In this context, ESA has supported microbial analysis in European spacecraft assembly facilities (Moissl-Eichinger et al., 2013; Stieglmeier et al., 2012). In particular, cleanroom maintenance procedures had a strong effect on what microbes were detected, with cleanroom areas predominated by potentially human-associated bacteria (Moissl-Eichinger et al., 2015). A group in Austria has recently used shotgun metagenomics and 16S rRNA amplicon sequencing and found that a loss of microbial diversity correlates with an increase in antimicrobial resistance, and this could aide in the "design" of microbiomes to minimize health risks to those living on human-made environments in space (Mahnert et al., 2019). Currently, the ISS microbial communities are considered to be highly similar to those present in ground-based confined indoor environments and are not deemed to pose a direct threat to human health (Mora et al., 2019).

ESA has also supported other works, such as research shedding light on the evolution and adaption of bacteria by interrogating the transcriptome of conjugative plasmid pAW63 (Van der Auwera and Mahillon, 2008), identifying bacterial communities on volcanic deposits of different ages (Byloos et al., 2018), and investigating extremotolerance and resistance of organisms to the Martian conditions (Jänchen et al., 2016). Additionally, adaptation of microorganisms to isolated environments was investigated by determining the microbial abundance of the surface snow surrounding the Research Base "Concordia" in Antarctica, considered an analog of extraterrestrial environments (Michaud et al., 2014). Further, microbial abundances after disturbance of salinity levels have been studied, wherein it was demonstrated that microbial community redundancy could restore the microbial diversity levels observed before the disturbance (De Vrieze et al., 2017).

Summary of European space omics contributions in plants

Arabidopsis thaliana is considered the plant model organism of choice for space biology. Although the contributions of NASA-supported space omic plant research have become increasingly important, multiple experiments have been performed by European researchers in collaboration with international colleagues over the last two decades. For example, the Seedling Growth project carried out on the ISS was aimed at unraveling the link between phototropism and gravitropism (main environmental cues driving plant development) in microgravity and reduced gravity environments, and how light conditions could be applied to overcome the deleterious microgravity-induced effects on plant development. Plants of different ecotypes of *A. thaliana* (Ler and Col-0) grew for 6 days in the European Module Cultivation System (EMCS), which allowed photostimulation of the seedlings with blue and red light, in addition to applying different g-forces by means of a built-in centrifuge. These studies found that microgravity produces (regardless of the ecotype and light wavelength) a reduction in the expression of genes related to photosynthesis and light detection, but an increase in the plastid and mitochondrial gene expression (Vandenbrink et al., 2019; Villacampa et al., 2021). A recent ESA-funded experiment was the first to reveal that the response of plants to microgravity is altered, not only in the number of de-regulated genes but also in the biological processes in which these genes are involved (Herranz et al., 2019). Specifically, microgravity

activates hormonal pathways responsible for proliferation and growth. Finally, these experiments demonstrated that red light photostimulation induces cell proliferation and ribosome biogenesis, two processes altered in darkness, in both microgravity and Martian gravity conditions. Further analysis of the differential response to red light photostimulation will assist in identifying genetic backgrounds advantageous for plants in future space missions (Manzano et al., 2020; Villacampa et al., 2021) (Tables S2 and S8).

In another experiment, Aubry-Hivet et al. (2014) used parabolic flights as an experimental platform to explore immediate transcriptional responses at early development stages of wild-type *Arabidopsis* and two auxin polar transport mutants (PIN2 and PIN3) in response to transient microgravity using microarray analysis. Their data suggest that the regulation of wild-type auxin-responsive genes is PIN-3 dependent. These results reflect different response mechanisms in gravity-perceiving columella cells (PIN3) and more distant zones mediating cell elongation responses (PIN2). These data were supported by previous work from the same group (Paponov et al., 2008), a terrestrial transcriptomic study of auxin-regulated genes associated with the biosynthesis, catabolism, and signaling pathways related to other phytohormones.

There was also the Genera A experiment, which consisted of growing *A. thaliana* plants for 12 days in EMCS on board the ISS to monitor global changes in the membrane proteome. In microgravity, proteins whose predicted function was related to auxin metabolism and trafficking were depleted in the microsomal fraction, while proteins associated with stress response, defense, and metabolism were more abundant. This approach highlights the key molecular processes affected during spaceflight and indicates that microgravity is perceived by plants as a stressful environment (Mazars et al., 2014a; b).

Finally, omics techniques have also been useful in optimizing standard operating procedures used in space and on the ground (e.g., in microgravity simulators). For example, transcriptomic analysis (using microarray) performed on the ground determined that the optimal procedure for plant preservation to study gene expression changes in simulated spaceflight conditions involves injection of RNAase inhibitors into the growth chambers while the plants are still on the rotors inside EMCS (Kittang et al., 2010). On the other hand, proteomic analysis was used to validate the ARADISH culture chamber designed to grow *A. thaliana* plants under simulated microgravity conditions, both on 2D clinostats and the random positioning machine. It focused on molecular biology approaches and thus avoided the lack of reproducibility and comparability observed in previous studies caused by the usage of a variety of different plant growth chambers and therefore different experimental conditions (Schüler et al., 2016).

To complement the experiments on plant seedlings, further research has taken advantage of cell culture in ground-based space simulation conditions to address fundamental mechanistic and functional questions. Several transcriptomic publications from a research group in Madrid (Herranz et al., 2013a; Kamal et al., 2019a, 2019b; Manzano et al., 2012) have shown that basic parameters of cell proliferation and cell division control are dysregulated under altered gravity conditions. Fundamental mechanisms, such as chromatin remodeling, are affected under these conditions, and should be tested in spaceflight experiments (i.e., true microgravity). Some of these transcriptomic studies have been complemented with proteomics analysis such as Differential in Gel Electrophoresis (DiGE) (Herranz et al., 2013b), while other groups have attempted to recapitulate these transcriptomic studies by exposing plant cell cultures to simulated intermediate partial gravity by centrifugation inside a clinostat or on board a parabolic flight (Fengler et al., 2016).

Summary of European space omics contributions in invertebrates (*D. melanogaster* and *C. elegans*)

Invertebrate model systems have been extensively used in spaceflight research since the first biological experiments were done in orbit, particularly in the 1980s where it was investigated how microgravity affects the oogenesis and development of embryos of *D. melanogaster* in the space shuttle during the Biorack experiment (Vernos et al., 1989). But, it was early in the 21st century when omics technologies could be used, with two European labs being the major players in the field (Professor Szewczyk's group at the time based in Nottingham (UK) for *C. elegans* and Professor Marco's group based in Madrid (Spain) for *Drosophila*). While the UK group has previously relied on Japanese missions, the Spanish group utilized a Soyuz Mission. Nonetheless, both teams carried out preliminary omics analyses (Herranz et al., 2005, 2007, 2008; Higashibata et al., 2006, 2007), including a comparative analysis of *D. melanogaster* and *C. elegans* spaceflight-induced gene expression responses (Leandro et al., 2007), before consolidating

results in several publications over a decade ago (Herranz et al., 2010; Selch et al., 2008) (Tables S3, S4 and S8).

In the case of *Drosophila*, extensive research in simulated microgravity facilities unraveled the effect/s of spaceflight on the transcriptome. Despite muscle and aging responses being evident, the contribution of the suboptimal environmental conditions due to hardware constraints (i.e., biorack type I container without forced ventilation) were hard to isolate from the true microgravity effects (Herranz et al., 2010). The *Drosophila* research landscape in Europe is quite different now, due to the premature close of the leading *Drosophila* lab. For some time, a collaboration with the plant microgravity lab, also in Madrid, facilitated European researchers to undertake a number of ongoing omics projects in simulated microgravity (Herranz et al., 2012, 2013a), and USA-based researchers have been populating the GeneLab database with *Drosophila* datasets (e.g. (Hateley et al., 2016; Hosamani et al., 2016; Ogneva et al., 2016)).

In the case of *C. elegans*, these nematodes offer many advantages compared to other experimental species such as the ease of handling/culturing, the microscopic size, and the opportunities for genetic manipulation (Etheridge et al., 2011; Higashibata et al., 2007). Regarding the effect/s of spaceflight on the transcriptome of *C. elegans* (Table S3), there have been several notable findings. For example, spaceflown *C. elegans* exhibits an altered transcriptional signature, demonstrated by the regulation of genes related to locomotion and regeneration processes (Higashibata et al., 2007, 2016). Additionally, gene expression changes in *C. elegans* are reproducible across spaceflights, with metabolic genes (e.g., genes regulated by insulin and TGF-β) demonstrating robust changes that may underlie the observed spaceflight-induced muscle decline in *C. elegans* (Higashibata et al., 2006; Selch et al., 2008). Surprisingly, differential expression analysis comparing *C. elegans* and *D. melanogaster* found very few genes that were similarly regulated in response to spaceflight, with only six genes decreased in expression across both conditions (Leandro et al., 2007), possibly reflecting different experimental designs. Interestingly, genes that are downregulated in *C. elegans* in response to spaceflight are known to impact physiology of *C. elegans* when knocked down on Earth (Honda et al., 2012). For example, the inactivation of genes related to neuronal or endocrine signaling extended life span on Earth (Honda et al., 2012). More recently, European-based scientists described molecular responses to spaceflight across multiple organismal models including *C. elegans*, resulting in the identification of shared and discrete transcriptional responses across the animal kingdom (Cahill et al., 2021). Microgravity and hypergravity transcriptomes have also been compared, highlighting transcriptional adaptations to altered gravitational load (Willis et al., 2020).

Summary of European space omics contributions in animal models

While a variety of experimental models have been used in the context of hyper/microgravity, fish are one of the rarer organisms. Of the identified studies, zebrafish (*Danio rerio*) have been the species of choice with both experiments conducted by the same European group based in Belgium (Aceto et al., 2015; Muller et al., 2010). This group has used *D. rerio* as a model organism to investigate bone formation and homeostasis, taking advantage of its small size, rapid development, easy maintenance, and sequenced genome (Aceto et al., 2015; Muller et al., 2010). Furthermore, the high degree of gene homology with humans suggests that *D. rerio* may be a suitable model organism to investigate other (patho)physiological phenomena in the context of hyper/microgravity in vertebrates (Aceto et al., 2015; Muller et al., 2010). The findings from these studies have collectively demonstrated that simulated hyper/microgravity can be successfully applied to *D. rerio* and such stimuli elicits gene level changes (Tables S5, S6 and S8).

Rodents, on the other hand, are a much more common model organism for spaceflight research. During the 1980–90s space shuttle era, rats became the model organism of choice for their long-standing use as a model for human physiology, and the ability to send sufficient numbers on missions to allow for quantitative biological research to be conducted. However, in today's biomedical research landscape, mice now account for ~61% of animals used in the European Union (EU) (versus ~12% for rats) (Commission, 2019). This is primarily due to their reduced housing needs and rapid breeding time, but most importantly the emergence of their amenability to genetic engineering. In line with terrestrial shifts in animal research, mice dominate the biological specimen payloads of spaceflights. Several missions have carried out experiments on mice on the ISS or on crewed missions.

Although most mouse investigations related to space adaptations have been developed by NASA, European researchers through ESA and their national research institutes have provided significant contribution

to the field through collaborative initiatives. The principal researchers involved in these contributions were typically from the Netherlands, Belgium, United Kingdom, Germany, Italy, and France (Acharya et al., 2018; Beck et al., 2014a; da Silveira et al., 2020; Fitzgerald et al., 2019; Fonte et al., 2019; Malkani et al., 2020; McDonald et al., 2020; Neutelings et al., 2015; Overbey et al., 2019a; Overbey et al., 2019b; Shinde et al., 2016; Tascher et al., 2017). Other contributions in the form of literature reviews and perspectives also involved researchers from Spain, Sweden, and the Netherlands (Afshinnekoo et al., 2020; Rutter et al., 2020). Most studies focused on gene expression, predominantly using microarray genome-wide gene expression analysis (e.g., Affymetrix platform) (Acharya et al., 2018; Beck et al., 2014a; Fitzgerald et al., 2019; Neutelings et al., 2015; Shinde et al., 2016) with only three studies utilizing RNA sequencing (da Silveira et al., 2020; Overbey et al., 2019a; Overbey et al., 2019b). Of note, we could only find a single European-contributed study that applied novel single-cell sequencing of circulating miRNA as well as genome-wide profiling of DNA methylation (Malkani et al., 2020). Based on the additional richness of data obtained in these studies, future studies should use cutting-edge technologies such as single-cell sequencing and imaging metabolomics to maximize the data obtained from these limited samples. Owing to the relatively limited supply of biological spaceflight tissues, these studies often combine data from human cell lines, the NASA twin study, as well as from various rodent tissues (i.e., cartilage, liver, bone, muscle, etc) and serum/plasma. Investigations into the field of regenerative medicine have been conducted to examine the impact of the space environment on mouse stem cells (Acharya et al., 2018; Shinde et al., 2016). Several studies focused on comparative analysis between human samples (e.g., blood serum/plasma) from the astronauts on different space missions. Additionally, data collected from research systems simulating reduced gravity (e.g., clinostat, random positioning machine, and parabolic flights) from mice and humans have been used to compare to samples collected after spaceflight on the ISS, Bion-M1 and Bion-M3, or the Rodent Research 1 and 3 space missions. Only two European-contributed studies so far have performed proteomic analysis (da Silveira et al., 2020; Tascher et al., 2017), while only a single metabolomics study was documented examining astronauts serum and urine (da Silveira et al., 2020). There is a notable absence of lipidomic studies and metagenomic analysis of the rodent microbiome, although some are starting to emerge (Alauzet et al., 2019, 2020; Tai et al., 2020). We predict that cellular lipidomic and microbiome studies will constitute a major axis of biomedical research to understand physiological adaptations during space travel and extraterrestrial settlement.

Summary of European space omics contributions in humans

Space biology studies have two broad aims, either to understand biological principles that can be used translationally to optimize human health in space travel or to explore basic biology using the unique environments provided by space missions. As plans for long distance spaceflight mature, concerns about the musculoskeletal effects of prolonged microgravity on astronauts have increased. Six studies have used bed rest as a ground-based model of microgravity to investigate its long-term effects. These utilized resistance training, aerobic training, dietary supplementation, and/or hypoxic conditions. All of these studies used genomic, transcriptomic, and/or proteomic analysis to identify fundamental changes in skeletal muscle or microbiota. Rullman et al. in 2016 (Rullman et al., 2016) and 2018 (Rullman et al., 2018) also analyzed microRNA expression. All used various countermeasures to mitigate the chronic changes associated with prolonged bed rest, which mimic those induced by prolonged exposure to microgravity (Chopard et al., 2009; Fernandez-Gonzalo et al., 2020; Rullman et al., 2016, 2018; Salanova et al., 2014; Sket et al., 2017). To investigate the mechanisms of muscle growth (hypertrophy), Lundberg et al. looked at the transcriptomic response to additional aerobic exercise to boost the hypertrophic response of resistance training (Lundberg et al., 2016). Importantly, these studies have applicability not only to spaceflight but also to people and patients on Earth who face similar musculoskeletal challenges due to (e.g.) aging, disability, or critical illness. In 2018, two studies looked at the effects of spaceflight *in vitro* on human cartilage cells (Lutzenberg et al., 2018) and *in vivo* in two astronauts (Rittweger et al., 2018). For the long-term vibration experiments on cartilage chondrocytes, there was no damage reported *in vitro*. In the human study, vigorous exercise appeared to be an effective countermeasure to spaceflight-induced muscle loss (atrophy), despite significant inter-subject variability (Tables S7 and S8).

In the SkinSuit study, microgravity was employed directly *in vivo* (Stabler et al., 2017). This study was performed to determine the effectiveness of a lightweight compression suit designed to provide head-to-foot loading to counteract spinal elongation during spaceflight. In parallel, the study also examined subgroups of skin microbiota, and how these changed in adaptation to the SkinSuit. While there was an overall effect to the microbiota signature, microbial diversity was maintained, and no pathogenic species were detected.

Further, the distinct microbiological "ISS signature" returned to baseline post flight. Research on the human skin microbiome very much remains a work in progress. A 2013 study identified Archaea, an ancient microbial lineage usually associated with extreme environments, present in the human skin microbiome (Probst et al., 2013).

Solar and cosmic radiation is another environmental hazard associated with spaceflight, particularly in deep space travel. In one study, a human endothelial cell line was subjected to linear energy transfer radiation and DNA damage with the changes in gene expression analyzed, which implicated the transcription factors E2F and nuclear factor (NF)-κB (Beck et al., 2014b). Another group focused on X-ray-based studies and described a transcription-based signature for dosimetry from human peripheral blood mononuclear cells irradiated with different doses of X-rays (Macaeva et al., 2016). The genes responsive to high-dose X-ray irradiation were identified, of which a significant number were genomic neighbors, giving insights into the gene control of cellular mechanisms responsive to radiation damage.

Grosse and collaborators (Grosse et al., 2012) investigated human thyroid cancer biology by studying cells under simulated microgravity conditions allowing for spheroid (3D) tissue culture, which is a better model of the *in vivo* microenvironment than that provided by the traditional 2D cell culture. Moreover, Ma et al. looked at the mechanisms responsible for tube formation by endothelial cells grown under simulated microgravity (Ma et al., 2013).

Microgravity has been utilized directly in other cell studies. One study (Versari et al., 2013) examined human umbilical vein endothelial cells cultured in low earth orbit and inferred that endothelial cell dysfunction was mediated through oxidative stress. Another study (Bradamante et al., 2018) conducted a stem cell differentiation experiment to determine how human bone marrow stem cells reacted to a prolonged exposure to microgravity in terms of growth and differentiation. These were also treated with vitamin D, which stimulates bone deposition, to determine its effects on the stem cell transcriptome. Analysis showed evidence of cell-cycle arrest occurring with a number of osteogenic gene markers (i.e., becoming quiescent after osteogenic differentiation) but without indications of adipogenesis, senescence, or apoptosis.

The chronic physiological changes seen in spaceflight recapitulate many of the classic changes of senescence (e.g., arterial stiffening and loss of bone mineral density). Calabria et al. examined microarray analysis of whole blood samples from either adults or older adults to observe changes in gene expression across all blood cell types (Calabria et al., 2016). Significant changes were found in the older adults, including decreased transcription of respiratory chain components, as well as changes in the expression of markers of inflammation/oxidative stress and immunosenescences.

ESA-funded investigators are also active in terrestrial fundamental biological research, crucially including DNA repair and cancer studies. Giang Ho et al. explored the key signaling molecules, RhoGTPases, and their expression patterns and their links with cancer progression (Giang Ho et al., 2011). Another study demonstrated that *POLD3*, the human ortholog of the yeast *POL32* DNA polymerase delta subunit, is required for cell cycle progression and processive DNA synthesis in a cyclin E overexpression model of DNA replication stress (Costantino et al., 2014). This implicates *POLD3* in break-induced replication, a mechanism that may be important in repairing damaged forks in cancer cells. A European-contributed study recently described how spaceflight impacts the immune system via transcriptomics (Buchheim et al., 2020). From profound basic science studies to directly applicable translational investigations for astronaut health, ESA funding has been, and continues to be, crucial in the nascent European human space biology community.

GEOGRAPHICAL DISTRIBUTION OF THE SPACE OMICS RESEARCH IN EUROPE

To gain an understanding of the geographical distribution of space omics research in Europe, we first visualized all European omics publications regardless of funding source (Figure 2A), and then secondly, visualized the ESA-(co)funded omics publications that we identified herein (Figure 2B).

In total, 94 European space omic publications were identified (regardless of funding source, see Methodological details). Of these, 75 acknowledged ESA (co)funding (Tables S10 and S11). While the biggest cluster of publications is located in Belgium around the cities of Mol and Mons, the greatest contributor of space omic research at a city level comes from Madrid (Table S11).

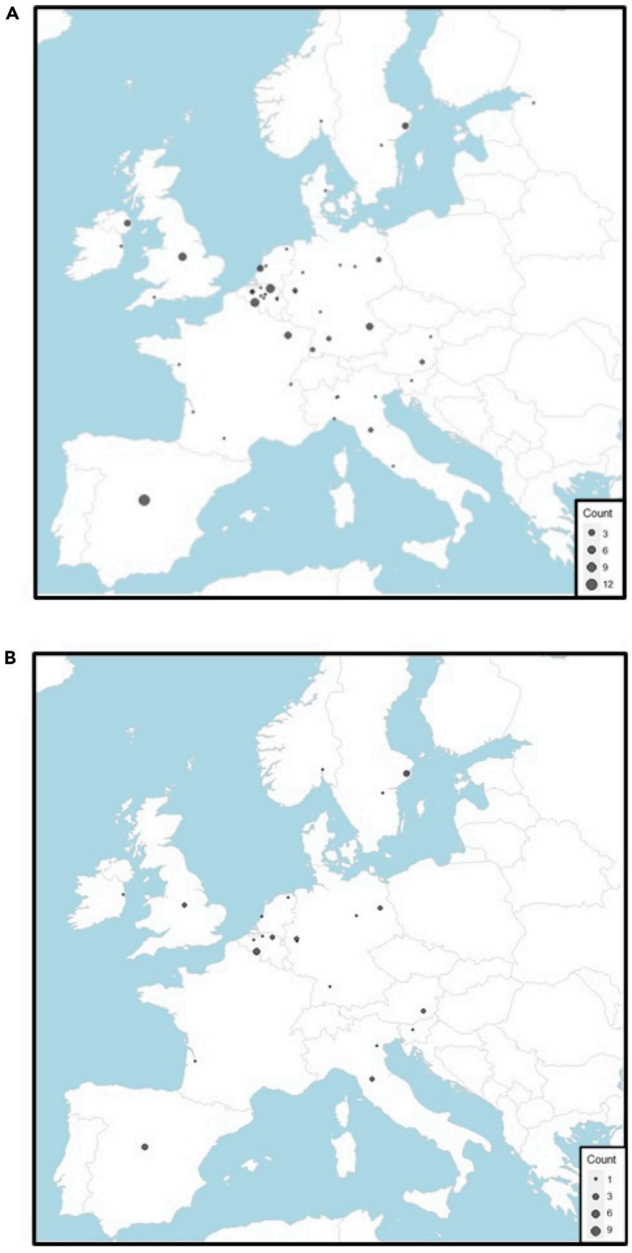

Figure 2. A map highlighting the European site of omics publications up until the first semester of 2020
(A and B) (A) and a map highlighting the site of omics publications co-funded by ESA between 2016 and the first semester of 2020 (B).

Belgium and Germany are the leading countries for space omics publications up until the first semester of 2020, with 24 and 17 publications each, respectively, followed by Spain (12), the UK (9), France (7), and Italy (7) (Figure 2A and Table S12). Regarding funding, Belgium, Germany, and Spain have the highest number of works (co)funded by ESA, with 23, 13, and 7 publications, respectively (Table S10). This shows that from the top five space omics- contributing countries in Europe, Belgian and German investigations are mainly supported by ESA, while Spanish studies are supported by both ESA-dependent and independent funding schemes. However, UK and French research was carried out with little ESA (co)funding. It is unclear whether this is the result of ESA policy, or whether it reflects research funded exclusively from national sources (which are not considered as ESA (co)funded), or if the project/s are funded by a completely different source.

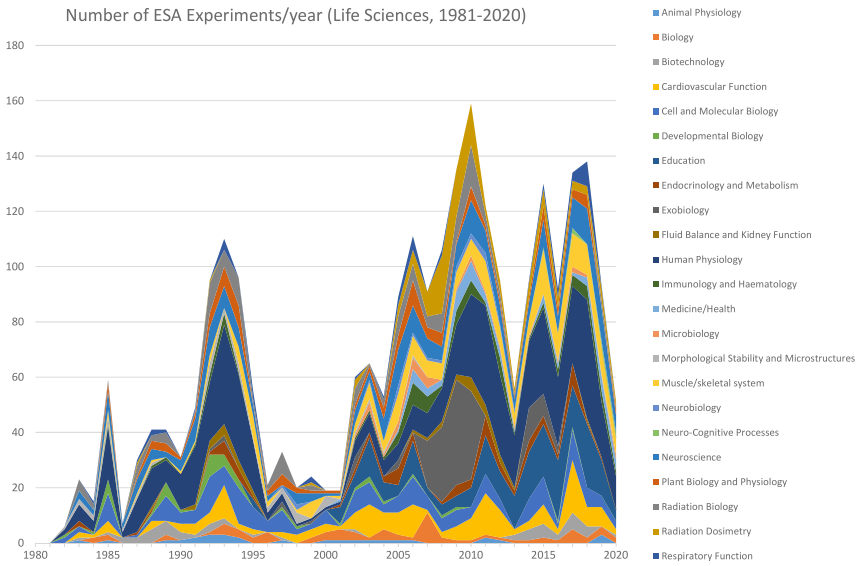

Figure 3. Number of ESA Life Science experiments performed per year by subdiscipline for the period 1980–2020 (data obtained from the ESA Erasmus Experiment Archive).

Focusing on publications which acknowledge ESA (co)funding and have been published in the last 5 years (2016–2020), Belgium and Germany still rank as the countries with the most ESA-(co)funded outputs, with 8 and 7 publications, respectively. Sweden was third with 4 publications, followed by Italy with 3 publications, then Spain, Austria, and the Netherlands with 2 publications each, and France, Ireland, Slovenia, and Norway with 1 publication each (Figure 2 and Table S12). The UK, the fourth biggest space omics producer in Europe, had no ESA (co)funding in the last 5 years, and France, at fifth place overall, acknowledged 1 ESA-(co)funded work (Tables S10 and S12). These differences in acknowledgment may reflect different national funding priorities.

Although this review is primarily focused on space omics research, we want to give a glimpse of the research performed in Europe across the whole biological sciences landscape. Focusing on the distribution of life science experiments per subdiscipline in the last 50 years (data extracted from the ESA ERASMUS archive, note that this information is general, non-specific to space omics (Figure 3), we found that the numbers vary depending on ESA priorities and platform development/availability at any time. For example, human physiology is typically stable in relative numbers, but exobiology/radiation topics were highly relevant in the period 2006–2010. Such variations in the topics/samples that are primarily selected to be flown by ESA may have an impact on the publications produced by the European space omics researchers, in comparison with the research teams working under NASA-flown experiments. The large number of experiments flown with ESA participation in Figure 3 highlights the importance of implementing common methodological approaches across independent experiments, so that results obtained from many different biological sub-disciplines can be harmonized and compared, in turn, capitalizing upon the scarce biological material recovered from spaceflight experiments.

Taken together, this data demonstrate that the European space omics research community will benefit from a coordinated funding strategy, more communication between European Union (EU), ESA, and national funding agencies, and more opportunities to interact with the broader space biology community. Indeed, these efforts are ongoing at ESA as reflected by the document ESA-BR269 "ESA Director General's Proposal for the European Space Policy" from 2007 (européenne and européenne, 2007) which states: "The EU, ESA, and their Member States have to continue to invest strongly to maintain leadership in space-based science". We advocate that spaceflight omics is the "frontier technology" and that it is crucial to expand the knowledge base and to develop new technologies and applications on space biology.

THE LIMITATIONS OF FUNDING SCHEMES FOR SPACEFLIGHT OMICS IN DIFFERENT ESA MEMBER STATES

Funding schemes for European and American researchers are very different and thus may have an impact on space omic research contributions. In the USA, space biology researchers usually obtain funding from NASA, which simultaneously acts as the funding agency and the spaceflight access agency. However, in Europe, the situation is much more complex. There is a clear separation between the funding source and ESA as the provider of the funding for spaceflight opportunities. On one hand, ESA covers all the costs related with the payload development (hardware) and the flight mission itself. But on the other hand, the funding for all research activities (e.g., reagents, equipment, and human resources) must be obtained from EU calls (e.g., Horizon Europe), national (research agencies or science ministry), or regional opportunities by the principal investigator and collaborators. Consequently, the research centers only receive this funding for consumables and personnel where it is subjected to overhead policies, and so principal investigators are more prone to cite these sources (i.e., EU calls) as the primary sources of funding, even if the flight/hardware-related costs covered by ESA are far higher. We, thus, elaborate on some ESA state member funding policies as an example of the variability across Europe.

In Spain, an ESA state member without a proper space agency, the Center for the Development of Industrial Technology (CDTI) is a Public Business Entity, answering to the Ministry of Science and Innovation, which fosters the technological development and innovation of Spanish companies, and it also runs as the ESA delegation in Spain. It is the entity that channels the funding and support applications for national and international research and development projects of Spanish companies, but is the link between ESA (paying for the payload ticket to space) and the national research programs (paying for the researcher's lab working costs). In Spain, it is usual to acknowledge the ministry first because the money comes directly from its research grant with a certain reference number. CDTI or ESA "co-funding" of Spanish research is invisible in most cases. When ESA is acknowledged, it is not unusual to occur without a grant reference. Despite these issues, 63.6% of the space omics work carried out in Spain acknowledges ESA (Table S10), and it is interesting to note that all the outputs came from Madrid (Table S11).

In Belgium, there is no dedicated national space agency, and the funding pipeline is similar as Spain. The Belgian Federal Science Policy Office is a federal government body responsible for research policy, including space-related research. It is recognized as the Belgium national delegation at the ESA Ministerial Council, taking place every 4 years (with intermediate sessions every 2 years). The Belgium delegation defines how much money they are willing to invest in various ESA programs for the coming 2–4 years. In short, acceptance/support by national delegation is a prerequisite for participating in an ESA program.

In addition to these complexities, some European countries have a dedicated national space research organisation (for example, German Aerospace Center (DLR) in Germany, UK Space Agency (UKSA) in the UK, or Center National d'Études Spatiales (CNES) in France) that may act as a complement to both ESA and state funding research agencies. The current UK funding landscape for space biology, including space omics, requires a double peer review system, whereby researchers must first apply for access to space through ESA. If successful, a second application to the UK Research Councils (UKRI) is then required to fund the science exploitation. Because space biology falls outside of UKRI's remit, the science case for such UKRI applications must typically center on Earth benefits (e.g., aging) to be successful. However, remit tensions between the UKSA and UKRI has led to a lack of consensus for which funding body should sustainably support space biology. Recently, UKSA has supported national payloads by funding both access to space and science exploitation, but the longevity of this approach is uncertain, and dependent on outcomes of government spending reviews and ministerial priorities to determine future UKSA budget portfolios. Outside of UK national payloads, upcoming ESA "Announcements of Opportunity's" (AO) within the E3P/SciSpacE envelope would benefit from omics-specific priority areas, but ESA mostly does not fund science costs, so bilateral funding agreements with national space agencies would be optimal (and *in lieu* of an ESA-managed omics facility). A recent report prepared by the UK Space Life and Biomedical Sciences Association called "Why Space? The opportunity for Health and Life Science Innovation" describes the broader space life sciences environment in the UK, how the country can benefit from its development, and the urgent need for dedicated funding and a better coordination between UKSA, UKRI, and ESA (Robson-Brown et al., 2021). In summary, funding space omics in the UK is currently disjointed and without a clear route to funding.

CNES is the main French institution in charge of funding spaceflight omics research. In fact, CNES finances space research at different levels. First, CNES provides financial support directly to researchers. To obtain it, researchers can submit a project to a CNES scientific committee once a year, and its acceptance allows researchers to apply for CNES funding. This funding is provided for 8-month duration and the amount is re-negotiated every year. This aid is used to finance laboratory experiments and the purchase of scientific equipment. It also allows the remuneration of staff and students in the form of fixed-term contracts, doctoral, and post-doctoral grants. Second, CNES funds spaceflight experiments by paying France'|'s contribution to ESA. Thus, France, as a member state of the European Agency, contributes to financing the experiments carried out in parabolic flights and on the ISS. The international opportunities for spaceflight experiments to which French researchers have access are the AOs from ESA, which give access to the ISS, parabolic flights, as well as to ground-based facilities (ESA-CORA-GBF), offering different microgravity simulators. The response to AOs is made in the framework of an international collaboration. French researchers also have the possibility of responding to the AOs of other international space agencies when these open their AOs to Europe. This is, for example, the case of NASA. In France, space research is funded solely by the CNES and researchers do not have access to other sources of national funding (institutional or private). In addition, space research requires a long-term investment, up to 10 years, incompatible with the requirements of other possible sources of funding (i.e., Agence Nationale de la Recherche), which are granted on average for 4 years.

The Swedish space program is mostly implemented through international collaborations where the Swedish National Space Agency (SNSA, or SNSB—Swedish National Space Board—until 2018) represents the contact entity for space cooperation. In other words, SNSA is a central governmental agency that can sign a contract within the space area on behalf of Sweden because it is its representative in the EU space program. The SNSA does not conduct its own research or development and most of its financial resources are dedicated to the ESA framework and bi-lateral cooperation. Around 70% of the Swedish space agency's budget is dedicated to international cooperation, while 20% is allocated to finance national research and development. In fact, SNSA is an administrative authority under the Ministry of Education responsible for government-funded national and international space operations for research and development. Swedish researchers, companies, and users of space applications can seek financial support from SNSA by submitting grant applications. These are subsequently reviewed by independent foreign auditors, and the board of the SNSA decides who will be awarded. In addition, SNSA invests in schools and outreach to the general public to train space-interested engineers, entrepreneurs, and researchers in order to promote and maintain Sweden as a successful space nation.

In summary, it is therefore quite challenging to find a similar "acknowledgement" section for all European researchers, due to the differential funding scheme/s for every party in EU. This complex scheme can have an impact on the productivity of each country in the space omics research field. If national and ESA programmes that support flight operations and science exploration could provide a predictable and sustainable funding landscape, it will facilitate long-term space omics projects, promoting collaboration among all eligible countries. Nonetheless, international collaborations can afford opportunities to circumvent some of the costs associated with omics analysis. For example, agreements with NASA GeneLab to perform RNA sequencing on space-flown samples have occurred, but the sustainability and scalability of this approach is likely to be low.

METHODOLOGICAL DETAILS

To locate ESA-(co)funded omics works, the list of relevant publications were sought by mining Web of Science using "European space agency", "Agence spatiale européenne", "Europäische weltaumorganisation", and "ESA" entered as the funding agency *and* the key search terms "transcriptomic" or "RNA sequencing" or "microarray" or "metabolomic/s" or "metabonomic/s" or "proteomics" or "2D-page" or "lipidomics" or "epigenomic/s" or "epigenetic" or "genomic" or "microbiome" or "metagenomic" or "metatranscriptomic" or "epigenomic" or "methylomic" or "epitranscriptomic", Tables S1–S7. In addition to these articles, the authors manually identified articles with a European-based space omics component, Table S8. Since we did not perform a systematic review, we apologize to those authors whose work we may have unintentionally omitted.

Geographical distribution of the space omics research in Europe was extracted from a reference list with all the articles cited on Tables S1–S8 created using Endnote 9 [https://endnote.com/] and all the PDF files

were downloaded. On Endnote 9, the information about authors, affiliation, country and city, year of publication, title, and author address were extracted to text files. Each of PDF file was converted to text file format and the acknowledgement and/or funding section were extracted. It was identified if the last two sections cited ESA. This was done by "pdftotext" [https://linux.die.net/man/1/pdftotext] which is available in most Linux distributions as well as Windows (via Xpdf) [https://www.xpdfreader.com/pdftotext-man.html]. Information from the two sources (Endnote text file and pdfs) was gathered and brought together via matching the title or short title with an in-house Python script. The resulting table containing all mentioned information was completed by getting the longitude and latitude of the cities the affiliation of the first author was located in. We used the WebAPI "nominatim" from OpenStreetMap [https://nominatim.openstreetmap.org/ui/about.html] to search for said cities via Pythons urllib[https://docs.python.org/3/library/urllib.html]. These geo coordinates were then used to create a map. The output table was checked manually and location and funding information was completed or corrected when necessary.

Using Excel (Microsoft Office), the articles were classified by location following the criterion: If the last author was based in Europe, their city was counted, if not the first author's city was counted. If negative for both cases, the senior European based author's city was counted. The articles were counted by country, Table S10, and by city, Figure 2A and Table S11. A subsequent filter was applied to identify articles published from 2016 that acknowledge ESA funding, Figure 2B and Table S12.

Map and location coordinates were acquired from Natural Earth (http://www.naturalearthdata.com/) and the maps were produced using R version 4.1 and packages ggplot2 version 3.3.5 and rgdal version 1.23.

LIMITATIONS

We acknowledge the likelihood that not all space omic-related studies (co)funded by ESA or produced by ESA state members are highlighted in our search. This may be for multiple reasons, including but not limited to i) ESA funding not being acknowledged, ii) the absence of a grant reference, iii) funding appearing in the acknowledgements (as opposed to the funding section), and/or iv) indirect support being provided by services (for example, consultation or the provision of equipment) as opposed to pure monetary support. Further, we know (through our Space Omics Topical Team network) that space omic-related studies have been conducted by scientists either located in/or collaborating with Europe, that have not appeared in our data-mining results due to either not explicitly stating ESA funding and/or not having experimental omic results (i.e., the outputs are narrative in nature). Owing to this, we have collated additional publications associated with the different model systems we covered herein, so a more extensive picture of ESA space omics research is captured. We, therefore, have included a non-exhaustive list of such studies (Table S8), which further demonstrate the utilization and application of omics to spaceflight within Europe.

CONCLUSIONS

ESA continues to be a major contributor to the field of space omics, mainly as a partner with other space agencies such as NASA and JAXA. An ESA-funded Space Omics Topical Team has been created to argue for the necessity of ESA and ESA state members to unify databases and resources and to promote the development of space omics to fulfill the role that Europe is expected to have in the field. This will also facilitate collaborations while preserving and promoting European-funded spaceflight experiments. In our view, there is a critical mass of European space biology researchers who can significantly contribute to space-related life science; the effectiveness of this depends on the prioritization and coordination of funding by ESA and complementary European funding bodies. In regard to the inclusion of studies without direct ESA (co)funding but being associated with ESA member states in Europe, our Space Omics Topical Team will make a number of recommendations in a follow up publication including the need for consistency/regulation in acknowledging ESA funding of the spaceflight opportunities and/or support with ground-based simulation experiments and the promotion of Findable, Accessible, Interoperable, Reusable (i.e., FAIR) compliant studies.

CONSORTIA

The Space Omics Topical Team aims to support the use of omics research among the ESA space biology community. The members of the Space Omics Topical Team are European-affiliated scientists landing at the Space Biology research field as Principal investigators from spaceflight biological experiments or as

bioinformatics experts. We worked to ensure gender balance in the recruitment of the members of the Topical Team and particularly in the key members responsible of coordinate the activity with the corresponding GeneLab Analysis Working Groups. We worked to ensure geographical diversity in the recruitment of members to represent the different state members of ESA in this committee. The list of members contributing to this article are Daniela Bezdan, Joseph Borg, Thomas Cahill, Eugénie Carnero-Diaz, Colleen S. Deane, Timothy Etheridge, Stefania Giacomello, Gary Hardiman, Raúl Herranz, Natalie Leys, Pedro Madrigal, Aránzazu Manzano, Felice Mastroleo, F. Javier Medina, Manuel A. Fernandez-Rojo, Keith Siew, Willian A. da Silveira, Nathaniel J. Szewczyk, Alicia Villacampa, Stephen B. Walsh, and Silvio Weging.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.103920.

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AUTHOR CONTRIBUTIONS

Conceptualization and methodology, CSD, WdS, and RH; software, data curation, and visualization, CSD, SW, and WdS; original draft preparation, CSD, WdS, and RH; subsection contributions, review, and editing, CSD, JB, ECD, TE, NL, PM, AM, FM, FJM, MAFR, KS, AV, and SG; academic English review, CSD, KS, and SBW; contribution to idea exchange, GH, NJS, SBW, and DB; project administration and editing, WdS and RH; funding acquisition, RH. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

DB is a cofounder of Poppy Health, Inc. and CSO of Yuri Gravity GmbH and declares additional affiliations at the NGS Competence Center Tübingen (NCCT), University of Tübingen, Tübingen and Yuri Gravity, Meckenbeuren, Germany. MFR declares additional affiliation at Diamantina Institute, The University of Queensland, St Lucia, QLD 4072, Australia. The authors declare there are no additional competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. One or more of the authors of this paper self-identifies as a member of the LGBTQ+ community. One or more of the authors of this paper self-identifies as living with a disability. We worked to ensure diversity in experimental samples through the selection of the cell lines. We worked to ensure diversity in experimental samples through the selection of the genomic datasets. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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