Boosting mitochondrial metabolism research in South America: building a case for a local *Drosophila* stock center

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Summary

***Drosophila melanogaster* is undoubtedly one of the most useful model organisms in biology. From a bioenergectics and metabolism point-of-view, its four discrete life cycle stages, each with particular nutritional and energetic demands, represent multiple powerful experimental systems in a single organism. Extensive resources are available for the community of *Drosophila* researchers worldwide, including an ever-growing number of mutant, transgenic and genomically-edited lines currently being developed and carried by stock centers in North America, Europe and Asia. Here, we provide evidence for the importance of stock centers in sustaining the substantial increase in the output of *Drosophila* mitochondrial research worldwide in recent decades. We also argue that the difficulties in transporting fly lines into South America has stalled the progression of related *Drosophila* research areas in the continent. Establishing a local stock center is the first step towards building a strong local *Drosophila* community that will contribute to the general field of mitochondrial research.**

Keywords – South America; stock centers; *Drosophila melanogaster*; mitochondria; metabolism

1. The historical expansion of *Drosophila* research and stock centers

Drosophila melanogaster is undoubtedly one of the most useful model organisms in biology. From helping to establish basic principles of genetics and evolution more than 100 years ago (Morgan 1910) to the determination of molecular mechanisms important to early animal development, activation of innate immunity and control of circadian rhythm, among other findings, *Drosophila* research has had a significant contribution to the science behind six Nobel Prizes in Physiology or Medicine (https://www.nobelprize.org/prizes/medicine). Most of this science, however, was only possible because of the resources that have been developed throughout the years and that are available for the community of *Drosophila* researchers worldwide, including, but not limited to, the ever-growing number of mutant, transgenic and genomically-edited lines that are carried by the stock centers in North America, Europe and Asia (https://wiki.flybase.org/wiki/FlyBase:Stocks). These facilities have the fundamental task of collecting, maintaining, creating and distributing *Drosophila* lines to any *Drosophila* lab in need to test the role of a particular gene, genomic region or genetic background in a biological phenomenon of interest.

Perhaps, one of the most important tools developed for *Drosophila* research has been the bipartite *UAS*/*GAL4* expression system, borrowed from the yeast *Saccharomyces cerevisiae* (Brand, Perrimon 1993). This system relies on the specificity of the GAL4 transcription activator, which is temporally and/or spacially expressed in particular transgenic fly lines called drivers, for the *cis*-regulatory *Upstream Activating Sequence* (*UAS*) that is fused to a genetic element of interest in a distinct transgenic line. When a *GAL4* driver line is then crossed with a line bearing an *UAS* construct, the offspring may overexpress or knock down (via RNA interference, RNAi) an endogenous gene, express an exogenous gene (from a different species) or a fusion construct to produce a fluorescently labeled protein, for example (Caygill, Brand 2016a; 2016b). Throughout the years, the *UAS*/*GAL4* system has been refined, expanded and/or combined with other systems to generate more advanced analytic tools, contributing to countless work describing the roles of genes related to human biology and disease, including those important in conserved signaling pathways, neural development of the brain, tumor progression and microenvironment, among other processes (Caygill, Brand 2016b; Lee, Luo 1999). The North American Bloomington *Drosophila* Stock Center (BDSC), the biggest center in number of stocks carried, currently lists approximately 8,300 *GAL4* driver, about 7,700 *UAS* non-RNAi and more than 13,000 *UAS*-RNAi stocks available (as of February, 2023, https://bdsc.indiana.edu/stocks/gal4/index.html, https://bdsc.indiana.edu/stocks/uas/index.html), illustrating the magnitude of experimental conditions possible with this system.

As for researchers working on other model systems, the *Drosophila* community has also benefited from the development and rapid expansion of the CRISPR technique (Gratz et al 2015) and other genome editing technologies, such that in about seven years the BDSC has accumulated and currently carries more than 4,800 related stocks for diverse purposes (as of February, 2023, https://bdsc.indiana.edu/stocks/genome_editing/index.html), including lines with the possibility of knocking out almost 3,000 genes by using a catalytically active Cas9, and of overexpressing almost 2,000 genes by targeting a nucleolytically dead Cas9 fused with the transcription activator VPR (dCas9-VPR) to a gene's endogenous promoter. Needless to say, most of the CRISPR and other genome editing lines are dependent on the *UAS*/*GAL4* system for proper function. Remarkably, these stocks were made available almost simultaneously to their publications to the scientific community through the BDSC, highlighting once again the central roles of stock centers in making important resources widely available.

We suspect that most Drosophilists (unwillingly perhaps) take stock centers for granted, so we sought to show if maintaining and distributing the abovementioned essential fly lines around the globe have made an impact on Nobel Prize-worthy and -not-as-worthy *Drosophila* research. We searched the Scopus databank (https://www.scopus.com) for worldwide publications related to *Drosophila* research throughout the years and correlated the results with the data on stocks carried and distributed by the BDSC within the same time span (https://bdsc.indiana.edu/about/history.html). Figure 1 shows how the growth in number of publications accelerated approximately 5.3 fold at about the same time that the BDSC started exponentially distributing its stocks. Importantly, that is just a few years prior to the first publication of the *UAS*/*GAL4* system (Brand, Perrimon 1993). Curiously, both the number of publications and the number of BDSC stocks distributed have plateaued in the last 10-15 years, suggesting a strongly intimate relationship.

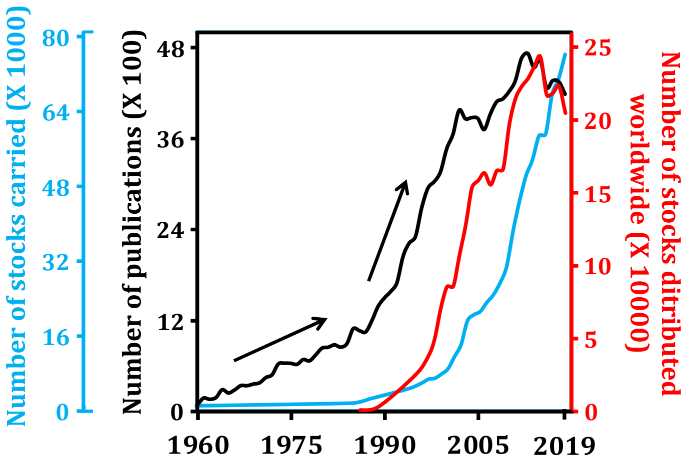

Figure 1. The historical increase in worldwide *Drosophila* publications is associated with the increased capacity of the BDSC to distribute its stocks. The number of publications trendline was constructed based on a search of the Scopus databank using the query "TITLE-ABS-KEY (Drosophila)". The arrows indicate the change in slope of this trendline after the late 1980s/early 1990s (from 1960 to 1986, linear equation y = 35.99x – 70464, R^2 = 0.98; from 1987 to 2003, linear equation y = 191.09x – 378764, R^2 = 0.99). The trendlines of the number of stocks carried and of stocks distributed worldwide by the BDSC were constructed based on the information available at https://bdsc.indiana.edu/about/history.html. Because of the impact of the COVID-19 pandemic on research output, data from 2020-2023 was not included.

We also noticed that the increase in the number of BDSC stocks carried and distributed trended similarly throughout the years, except that the former lagged behind a few years and has not plateaued yet (Figure 1). The implementation of user fees in 1995 (https://bdsc.indiana.edu/about/history.html) coincided with the beginning of the expansion in the BDSC's capacity to carry new stocks, allowing it to reach close to 80,000 stocks in 2019. Thus, it is apparent that the more BDSC distributed stocks, the more it contributed to *Drosophila* research output and the more revenue it generated to invest in its own capacity to carry the new technologically advanced research tools Drosophilists were continuously creating. We suggest this in turn produced a positive feedback loop with more distribution capacity and more research output by the community. We also speculate other stock centers have made similar impact on research output, perhaps not as much wordwide, but more locally due to their more limited size in number of stocks carried. In conclusion, together, stock centers are indispensable for the success of the local and worldwide *Drosophila* community in the short- and long-term future.

2. The problem of stock distribution to South America, especially Brazil

For many years, we and other Brazilian *Drosophila* researchers have used the services provided by the North American, European, and Asian stock centers to obtain the fly lines necessary for our work. We have encountered high costs and a tremendous amount of bureaucracy, which have stalled our work significantly or prevented it from occurring even when met with the proper legal documentation. Assuming that a local Brazilian flylab is already authorized by the National Technical Committee on Biosafety (http://ctnbio.mctic.gov.br) to work with level 1 genetically modified organisms (GMOs), a simple request to the Local Biosafety Committee of the research institution to authorize the import of fly lines would in theory be sufficient, paperwork-wise. However, the country's customs authorities do not recognize this documentation only; they rely primarily on documentations from the Ministry of Agriculture, Livestock and Food Supply (MAPA), responsible for supervising the entry of live organisms into the country.

The Universal Postal Union Convention Manual (https://www.upu.int/UPU/media/upu/files/UPU/aboutUpu/acts/manualsInThreeVolumes/actInThreeVolumesManualOfConventionEn.pdf) is used by *Drosophila* stock centers as guidance for shipments of transgenic or non-transgenic flies to various countries. Although its Article 19 states that "flies of the family Drosophilidae for biomedical research exchanged between officially recognized institutions" are items permitted to be shipped internationally, each country can impose its own set of rules. In Brazil, MAPA's Normative Instruction No. 36, of October 11, 2006 (http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=recuperarTextoAtoTematicaPortal&codigoTematica=1265040) details the operational procedures for international agricultural surveillance at ports of entry, without any mentioning of organisms relevant to biomedical/biological research. In practical terms, *Drosophila* researchers who need to import lines for basic research must apply for the same authorizations that a large importer of agricultural goods would; a complex, arduous and bureaucratic task (details at https://sistemasweb.agricultura.gov.br/pages/SEI.html). According to the responsible office in MAPA, the official processing time of the paperwork is reasonable, but in practice we deal with professionals who are overloaded with work, and untrained to assess the risks of harmless *Drosophila* lines, and often unable to approve our import requests in a timely fashion. In our experience and that of other colleagues, the proper documents can take from 30 up to 90 days to be issued.

Once all import documentation is approved, Brazilian Drosophilists then face the "obstacle" of delivery. *Drosophila* stock centers usually ship their stocks in vials adequately packed in boxes, providing an appropriate environment for the flies for about 2 weeks. If using standard shipping, which in the case of BDSC is via the United States Postal Services (USPS), often the stocks only arrive at labs in Brazil in 30-60 days. Even with the correct import documents, the packages remain awaiting customs approval for an excessive amount of time, often encoutering warehouse temperatures well above or below what is ideal for fly survival. And because USPS relies on the Brazilian Postal Services (Correios) once the package arrives in Brazilian territory, USPS tracking stops when the package leaves the United States and Correios never register it in its own tracking system. Thus, we often receive dead flies by mail without the option of tracking or inquiry during shipping and delivery.

Alternatively, a Brazilian *Drosophila* researcher can request shipment via an express delivery specialist, such as FedEx®, which often solves the problem of receiving dead flies by mail. However, this comes at great monetary costs, as the shipping charges can reach three times that of a purchased stock. For example, in our last purchase of BDSC stocks, we spent USD$ 15.00-25.00 per fly line for research and more than US$60.00 in shipment via FedEx®. These numbers may not seem absurd to a researcher in North America or Europe, but in our opinion, the problem becomes more apparent when put into the perspective of the current science funding situation in Brazil: main Federal grants pay no more than ~USD$ 1,760.00 (BRL$ 9,167.00) per year per principal investigator (http://resultado.cnpq.br/2539505081395270; https://www.nexojornal.com.br/colunistas/2021/Qual-o-limite-de-resiliência-do-cientista-brasileiro) and the monthly stipends of PhD students with Federal funding have been stuck at ~USD$ 420.00 (BRL$ 2,200.00) for almost a decade (Jordão 2019; https://www.nexojornal.com.br/grafico/2022/03/23/Bolsas-da-Capes-e-CNPq-completam-9-anos-sem-reajuste). Finally, upon arrival at customs, the package can still be taxed incorrectly as commercial goods. On one occasion, we received a single fly line as a donation from a researcher in Japan, who voluntarily paid for all FedEx® shipping costs, and we were still charged ~USD$ 170.00 (BRL$ 900.00) in obscure fees by the Brazilian customs to have the entry of the flies into the country approved. These fees appear to be randomly applied, as there is no clear way of knowing beforehand if a fly shipment will be tariffed upon arrival at any Brazilian port of entry.

Some science funding agencies do offer a broker service that facilitate import of scientific research material, including GMO animals. The Sao Paulo Research Foundation (FAPESP, https://fapesp.br/en), for example, is one of these agencies, but using this resource (which is provided only to FAPESP grantees) is truly only feasible when the total import costs are at least USD$ 1,000.00, as FAPESP needs to offset the costs of customs clearance. For a single Drosophilist, this amount would require importing 40-65 fly lines all at once (based on the BDSC stock prices as of February, 2023 - https://bdsc.indiana.edu/order-accounts/fees/index.html), which in turn would take extensive research planning beforehand. In summary, in Brazil, we cannot promptly order a few stocks and receive them one or two weeks later to complete an experiment, like *Drosophila* researchers in developed countries usually do. In the best case scenario, if research funding is available to overcome the high costs, and all steps of the import process described above are completed smoothly, fly stocks will legally reach Brazilian labs in no less than 5 months. Needless to say, some of us eventually obtain fly stocks in an illegal way, helped by traveling colleagues and friends that are willing to carry a few fly vials with their personal belongings in the checked luggage. Or we may receive a donation of flies that have (luckily) made into the country on a previous occasion and are being propogated locally in a colleague's lab, a practice that is prohibited by most stock centers, since it negatively affects their revenue. It is reasonable to assume *Drosophila* researchers from other South American countries also have elevated shipping costs, difficult customs clearance processes, and/or low science funding, making fly research difficult anywhere on the continent.

Directly quantifying how disruptive the high costs and bureaucracy are for Brazilian/South American *Drosophila* research is impractical, since nuances in the relatively low budget for scientific research in these countries likely have a much bigger impact on research output. We then sought to again correlate yearly growth in number of publications by Drosophilists from South America and Brazil alone, and the number of BDSC stocks distributed to the continent. Figure 2 (*left panel*) shows that since 1990 publications have increased continuously in a linear fashion, with modest slopes. We were only able to obtain data on the BDSC stock distribution to South American countries for the last 20 years, but this appears sufficient to indicate a general tendency for increased distribution to the continent in the last ~10 years. On the other hand, no tendency for increased stock distribution to Brazil alone was observed (Figure 2, *right panel*), indicating that the country's researchers may not be taking advantage of the numerous recently developed technologies, as described in the previous section, or that other stock centers may be the primary suppliers of lines to Brazilian Drosophilists. We find that last option improbable, since the European Vienna *Drosophila* Resource Center (VDRC), perhaps the second largest stock center in the world, only lists two locations in Brazil which are their current shipping destinations of fly lines (https://www.viennabiocenter.org/vbcf/vienna-drosophila-resource-center/). In summary, we cannot show that the same strong relationship observed between BDSC stock distribution and the worldwide output of *Drosophila* research exists in South America, especially in Brazil. We speculate that the "steady, but slow" increase in research output from South American Drosophilists comes from studies of naturally occurring local Drosophilidae populations, which are rich in the continent (Döge et al 2008; da Mata et al 2008; Bizzo et al 2010; Poppe et al 2014). This unfortunately only allows certain areas of biology to progress more substantially, such as ecology and population genetics, an idea that is supported by the number and type of funded projects using *Drosophila* by FAPESP (Garcia et al 2021).

3. Limited output of *Drosophila* mitochondrial metabolism research from South America

From a bioenergetics and metabolic perspective, *Drosophila* provides several powerful experimental systems in a single model organism. It has four distinct life cycle stages, each with particular nutritional and energetic demands. At the embryo stage, the organism relies solely on the resources maternally provided in the egg to allow the recurrent cycles of nuclear division that precedes cellularization and determination of body segmentation. Similarly to early development in mammals, mitochondrial biogenesis is negligible at this stage, so that basically the same number of maternally-inherited mitochondria is distributed among the newly formed tissues as development proceeds. At its end, the number of organelles per cell is dramatically lower than in the egg/early embryo, with possible bottleneck effects for the development of tissues/organs carrying a particular mitochondrial DNA haplotype in a heteroplasmic condition. Interestingly, midway through embryogenesis, the new organism's expression of glycolytic genes is activated (Tennessen et al 2014; Tennessen, Thummel 2011), indicating the importance of carbohydrate metabolism at this phase.

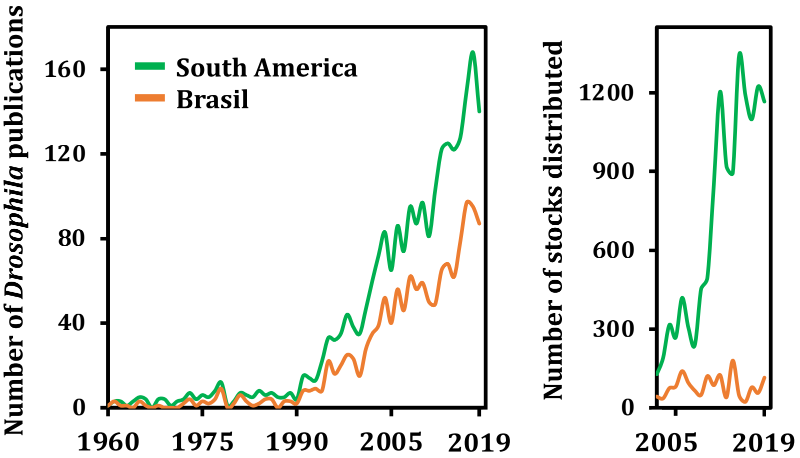

Figure 2. Limited BDSC stock distribution to South America and correlated *Drosophila* publications. The number of publications trendlines were constructed based on a search of the Scopus databank using the queries "AFFILCOUNTRY (Brazil OR Argentina OR Colombia OR Peru OR Bolivia OR Chile OR Venezuela OR Paraguay OR Uruguay OR Ecuador) TITLE-ABS-KEY (Drosophila)" and "AFFILCOUNTRY (Brazil) TITLE-ABS-KEY (Drosophila)". From 1990 on, linear equations were, respectively, $y = 5.02x - 9981$ ($R^2 = 0.95$) and $y = 2.97x - 5909$ ($R^2 = 0.92$). The trendlines of the number of stocks distributed to South America and Brazil alone were constructed based on the information made available by the BDSC to the authors. These numbers must be seen as approximations of the real number of stocks shipped to the continent due to four main reasons: 1) prior to 2011, BDSC occasionally reused account numbers, which may have produced confounding numbers; 2) the numbers do not include stocks that died en route and had to be reshipped; 3) the numbers reflect the current location of the primary BDSC account holder (the principal investigator), including from a time during which the account holder may have been based on a non-South American country; and 4) they include stocks shipped to non-South American countries, but which were ordered from a BDSC account for which the holder is based on a South American country. Because of the impact of the COVID-19 pandemic on research output, data from 2020-2022 was not included.

The larval stage is marked by pronounced food consumption, growth and biomass accumulation (up to 200 fold in ~4 days at 25 ºC, Jacobs et al 2020), which occurs in three distinct sub-stages referred to as instars. At the same time that levels of lactate dehydrogenase peaks, indicating the importance of aerobic glycolysis (Li et al 2017), larval mitochondrial metabolism is essential and primarily anabolic, providing tricarboxylic acid (TCA) cycle intermediates to the synthesis of fatty acids, nucleotides and amino acids, among other molecules, and allowing storage of fuel and building blocks for the next phase of development, the pupa (Jacobs et al 2020). At the pupa stage, an extensive tissue rearrangement called metamorphosis takes place, starting with histolysis of almost all larval tissues and ending with the biogenesis of organs of the future adult fly. During this time, the individuals must rely solely on the lipids, carbohydrates, proteins and other nutrients stored at the larval stage, and balance the breakdown of an organism physiologically adapted to growth with the building of a new one that is intensively metabolically active, the adult.

The adult is an incredibly athletic animal, demanding relatively high amounts of food to provide enough fuel for ATP production in its flight muscles via mitochondrial oxidative phosphorylation (OXPHOS). In fact, mitochondria are so important for insect flight that they can occupy a third of the total volume of muscle cells (Levenbook, Williams 1956). And as in other animals, flight and locomotion is also highly dependent on a functional neurological system that can coordinate muscle and other activities. Mitochondrial metabolism is also key for neuronal function, and in this case not only because of ATP production via OXPHOS, but also because of its role in the synthesis of neurotransmitters like glutamate and γ-aminobutyric acid (Rowley et al 2012). In females, the ovaries provide perhaps one of the best systems to study mitochondrial biogenesis, as new eggs are constantly being produced, allowing a single individual to lay up to 500 of them throughout its lifetime (Demerec 2008). The male testes, on the other hand, provide a fascinating system for the studies of mitochondrial dynamics, as mitochondria are continuously being remodeled as spermatogenesis progresses. They start as several distinct small units in the germline stem cells, are fused into a single, immense mitochondrial mass called the Nebenkern in early spermatids, and later split into two units that serve as a plataform for microtubule assembly and spermatid/spermatozoid tail elongation (Noguchi et al 2011). Curiously, the *D. melanogaster* genome has a significant number of paralogues encoding central and mitochondrial metabolism genes whose expression is restricted to the testes (http://flybase.org), indicating an interesting area of study in which one could correlate mitochondrial morphology and metabolic function, and show how metabolism genes evolve by duplication, sequence divergence, and tissue specialization.

With this diversity of experimental systems to investigate metabolism and bioenergetics, combined with the versatile research tools available and the established distribution capacities of stock centers, one would expect that the number of publications related to *Drosophila* mitochondrial metabolism research was to trend similarly to that related to general mitochondrial metabolism research, at some point after ~1993. This is in fact what we observed by exploring the Scopus databank for worldwide publication data. It is also clear that the overall contribution of *Drosophila* research to the general field

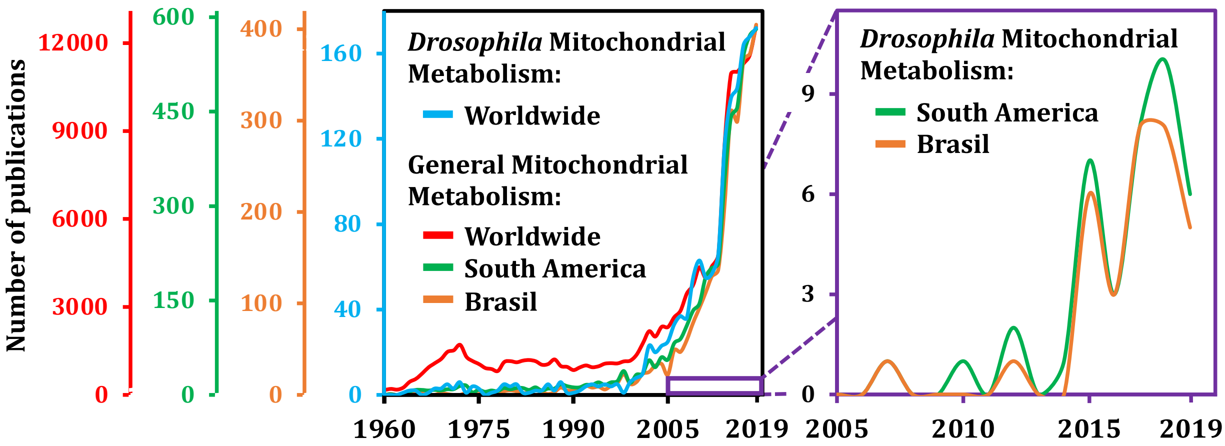

Figure 3. Limited output of *Drosophila* mitochondrial metabolism research from South America. The number of publications trendlines were constructed based on searches of the Scopus databank using the following queries: "TITLE-ABS-KEY (Drosophila AND mitochondr* AND metabolism)"; "TITLE-ABS-KEY (mitochondr* AND metabolism)"; "AFFILCOUNTRY (Brazil OR Argentina OR Colombia OR Peru OR Venezuela OR Chile OR Paraguay OR Uruguay OR Bolivia OR Ecuador) TITLE-ABS-KEY (mitochondr* AND metabolism)"; AFFILCOUNTRY (Brazil) TITLE-ABS-KEY (mitochondr* AND metabolism)"; AFFILCOUNTRY (Brazil OR Argentina OR Colombia OR Peru OR Venezuela OR Chile OR Paraguay OR Uruguay OR Bolivia OR Ecuador) TITLE-ABS-KEY (Drosophila AND mitochondr* AND metabolism)"; AFFILCOUNTRY (Brazil) TITLE-ABS-KEY (Drosophila AND mitochondr* AND metabolism)". Because of the impact of the COVID-19 pandemic on research output, data from 2020-2022 was not included.

of mitochondrial metabolism has been nevertheless very timid (Figure 3, *left panel*). The trends in publications on general mitochondrial metabolism in South America and in Brazil alone are also very similar to that seen worldwide, except for a delay of a few years in the former (Figure 3, *left panel*). The contributions of Brazilian mitochondrial researchers to the field in the last 20 years have been substantial and are highlighted elsewhere (Vercesi, Oliveira 2018). However, analyzing the output of *Drosophila* mitochondrial metabolism research from South America showed that no publication was recorded before 2007 and that an average of 3 articles on the subject were published per year since then (Figure 3, *right panel*). It appears that since 2015 there has been a substantial increase in the number of publications per year, mostly driven by researchers in Brazil, but the numbers are still too low to indicate a clear growth tendency and predict the overall contribution of South Americans to the field of *Drosophila* mitochondrial metabolism.

4. Conclusions: a way to boost mitochondrial metabolism and general *Drosophila* research in South America

Here, we have shown the historical intimate relationship between stock distribution by the BDSC and the increase in worldwide *Drosophila* research output, as measured by the yearly number of publications registered in the Scopus databank since 1960. We have also shown that BDSC stock distribution to South American countries is limited and that it appears to have no clear relation with the number of *Drosophila* publications by South American researchers. When we evaluate only publications on *Drosophila* mitochondrial metabolism, our primary area of interest, the numbers are simply too low to infer any clear pattern or correlation, even though mitochondrial metabolism and redox biology in South America, especially in Brazil, are firmly established fields of research. We also presented anectodal evidence that one of the most important impediments for the expansion of *Drosophila* research in Brazil is the bureaucracy and high shipping costs associated with the importation of GMO fly lines into the country. Small individual *Drosophila* research groups usually have low leverage, such that dealing with fly line imports often becomes too much of a burden, even when research funds are available. This may ultimately discourage a lab from starting to use *Drosophila* as their main model organism for biomedical/biological research, despite the myriad genetic tools this organism offers.

The difficulties of importing reagents/materials for scientific research in Brazil is not exclusive to Drosophilists. This is a long-lasting problem that has affected scientists in all areas, caused by an inherently inefficient customs service (https://revistapesquisa.fapesp.br/en/supply-side-research-constraints/). We find it improbable that this system will be reasonably improved in the near future, or that our currently small community of *Drosophila* researchers will gain enough leverage to establish a channeled fly import process through a customs broker service. We first need to make the community grow, but this cannot be achieved without a fast way to receive the fly lines of interest. Therefore, to boost mitochondrial metabolism and general *Drosophila* research in Brazil, and consequently in all South America, we propose the establishment of a local stock center that will be able to maintain and distribute important fly stocks all over the continent affordably and reliably. Given the trajectory of BDSC stock distribution and its correlation with the increase in worldwide *Drosophila* publications (Figure 1), we envision that a local South American stock center will likely be the most important step towards the construction of a foundation upon which the *Drosophila* community will expand significantly in a matter of years. We will also trust our currently small community to advocate for *Drosophila* research in their own local workplaces and in national and international conferences.

The establishment of a local stock center will come with no shortage of challenges. The development of new genetic tools, such as a collection of *UAS* non-RNAi, *UAS*-RNAi or of CRISPR lines that would be exclusive to the new stock center, would demand a tremendous effort and a significantly high investment. Instead, we envision the possibility of creating a "branch" of the North American, European and/or Asian stock centers, in which some of their already well-established stocks would be made available once to our future South American stock center, to be kept locally, indefinitely and always ready to be shipped promptly, for an affordable price. All the importation troubles described above would then be concentrated and only occur once every one or two years, when the local stock center would expand by receiving new stocks from their original sources. This expansion would be done as democratically as possible by consulting the *Drosophila* community about their fly lines of interest. Some of the well-established stock centers may find this proposition to be not worth pursuing, since most of the revenue generated by stock distribution by the putative future South American stock center would remain locally; only a fraction of the revenue would then be paid to the center in which the stocks originated, as some sort of "royalty" fee. We believe that in the long run distributing stocks to South American labs mediated by a local "branch" will pay off to the original stock centers; the alternative would be to continue receiving a minimal number of orders (or none at all) and very little revenue from South Americans due to either the small size of our fly communities or the impracticalities of our importation systems.

Obviously by being based in Brazil and assuming that the Brazilian *Drosophila* community is likely the largest in South America, we envision this future stock center being established here, but this may impose other types of challenges. Brazil is arguably the number one country in enforcing its rights under the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity (https://treaties.un.org/pages/ViewDetails.aspx?src=IND&mtdsg_no=XXVII-8-b&chapter=27&clang=_en#EndDec), because it is legitimately concerned about pharmaceutical industries and other countries exploiting its natural resources while seeing no economic benefits from their use. The country adopts a broad interpretation of "natural resource", but at this point it is not clear if that comprises research organisms such as *Drosophila*. In theory, it is possible that the authorities may view any fly line originated elsewhere that has been propagated in a lab in Brazil as subject to the Nagoya Protocol, even if it is unchanged genetically. This may be an impediment/discouragement for stocks to leave the center in Brazil towards other South American countries, or at least, the issue of GMO flies originated elsewhere being under the Nagoya Protocol in Brazil would have to be discussed and clarified with government authorities prior to the shipping of the first stocks out of the country.

The new stock center, being in Brazil or anywhere else in South America, would have to be attractive to researchers, showing the advantages of using *Drosophila* as their main or additional model organism. Once again, we will also have to count on our community to advocate for fly research and help show such advantages. But, in our opinion, the abundance of genetic tools provided by *Drosophila* will not necessarily tip the balance towards a switch in model organisms by a research group if it does not come associated with lower costs. We are currently quantifying how much it would cost to perform several different types of experiments commonly executed in a molecular biology lab in Brazil using *Drosophila* and human cells in culture, with the intention to also show monetary advantages for the former and to help create attractiveness. Nevertheless, once established, the putative new South American stock center should rely primarily (or even solely) on funds from universities, research institutions and funding agencies during its first years, balancing the costs of stock maintenance and distribution and providing fly lines at a minimal price (or even free of charge) to local Drosophilists, making the use of *Drosophila* very appealing. Government funding of the putative stock center will then be key, as it is even for the well-established BDSC (https://bdsc.indiana.edu/about/funding.html) and VDRC (https://stockcenter.vdrc.at/control/main). One frequently hears that flying is most often the fastest and sometimes the cheapest way to reach a destination. For South American mitochondrial and other researchers, we believe it is time to “fly”!

Abbreviations

BDSC	Bloomington *Drosophila* Stock Center	OXPHOS	Oxidative Phosphorylation
dCas9-VPR	nucleolytically dead Cas9 fused to VPR	RNAi	RNA interference
FAPESP	Sao Paulo Research Foundation	TCA	Tricarboxylic Acid
GMO	Genetically Modified Organism	*UAS*	*Upstream Activating Sequence*
MAPA	Brazilian Ministry of Agriculture, Livestock and Food Supply	USPS	United States Postal Service
		VDRC	Vienna *Drosophila* Resource Center

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