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PORTADA/ COVER

Nuestra portada combina imágenes de los temas abordados en los artículos de este ejemplar.

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Illustration of the diversity of topics covered in this issue.

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UNIVERSIDAD INTERAMERICANA DE PUERTO RICO
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MENSAJE DEL RECTOR
DR. RAFAEL RAMÍREZ RIVERA

Es un honor presentar a nuestros lectores el volumen 7 de la revista *Inter Scientific*. Agradezco a todos los colaboradores por su compromiso con la investigación y la difusión del conocimiento en un tiempo tan difícil, que nos ha tocado vivir de pandemia de COVID – 19. Sin embargo, esta situación de salud mundial nos permitirá reflexionar sobre el futuro biológico, ecológico, económico, social, entre otros, para construir nuestra nueva realidad.

Reconocemos a los profesores, estudiantes y a la Universidad Autónoma de Guadalajara (UAG) School of Medicine, Programa Internacional de Medicina y al Programan Nacional de Medicina por publicar en nuestra revista. Esto nos presenta ese espacio innovador que nos acerca al intercambio intelectual de profesores y estudiantes que se beneficiarán de ese enriquecimiento científico y tecnológico en el que está inmerso el proceso de enseñanza aprendizaje en las sociedades que se transforman y se internacionalizan.

Les invito a sumergirse en una lectura apacible y de análisis crítico.

MESSAGE FROM THE CHANCELLOR
DR. RAFAEL RAMÍREZ RIVERA

It is an honor to present our readers with the seventh volume of *Inter Scientific*. Thanks to all the collaborators for their compromise with research and the spread of knowledge in such a difficult time when we face the pandemic caused by COVID-19. However, this worldwide health issue has allowed us to reflect on the biological, ecological, economic and social future, among other aspects, in which we will build our new reality.

We recognize the faculty, students and the School of Medicine of the Universidad Autónoma de Guadalajara (UAG), International and National Medicine Programs, for publishing in our journal. This represents a space for innovation that brings us closer for intellectual exchange among faculty and students that will benefit from the scientific and technological enrichment in which the teaching-learning process of the societies that transform and internationalize are immersed.

I invite you to immerse yourself in a gentle and critical reading.

MENSAJE DE LA DECANA DE ASUNTOS ACADÉMICOS DRA. KAREN WOOLCOCK RODRÍGUEZ

La revista Inter Scientific lleva publicándose de forma ininterrumpida desde el año 2014. En esta ocasión la séptima edición se publica una versión electrónica y recopila las investigaciones y otras gestiones científicas realizadas antes de la pandemia del COVID-19. Los esfuerzos por continuar produciendo nuevos conocimientos es parte de la misión de la Universidad y nuestro Recinto cuenta con los docentes, colaboradores y colegas que trabajan duro para aportar información que impacta distintos campos de la ciencia. En esta edición se destaca muy particularmente los esfuerzos de nuestros estudiantes guiados por docentes comprometidos, por realizar investigaciones estructuradas en nuestra unidad académica. Además, se incluyen publicaciones de científicos de otras Instituciones que analizan el comportamiento de especies de la fauna de Puerto Rico, que definidamente enriquecen nuestros conocimientos y nos concientizan sobre la belleza e importancia de la interacción entre las especies. Finalmente, se incluye por primera vez un resumen comprensivo realizado por un grupo de profesionales clínicos, que evalúa los datos producidos por la investigación. Les invitamos a la lectura de tan variada publicación y le exhortamos a que continuemos creando los espacios para promover la investigación científica, particularmente entre los estudiantes que serán nuestros líderes del futuro.

MESSAGE FROM THE DEAN OF ACADEMIC AFFAIRS DR. KAREN WOOLCOCK RODRÍGUEZ

Inter Scientific has been published uninterruptedly since 2014. In this occasion the seventh edition is published as an electronic version that presents research and other scientific endeavors conducted before the pandemic caused by COVID-19. The efforts to continue generating knowledge are fundamental part of the mission of our University; our Campus has the faculty, collaborators and colleagues that work hard to provide information that influences many fields among the sciences. In this edition, the efforts of our students guided by committed professors to carry out structured research in our academic unit are particularly highlighted. In addition, publications by scientists from other Institutions that analyze the behavior of species of the fauna of Puerto Rico are included, which definitely enrich our knowledge and make us aware of the beauty and importance of the interaction between species. Finally, a comprehensive summary by a group of clinical professionals is included for the first time, evaluating the data produced by the research. We invite you to read such a varied publication and we urge you to continue creating spaces to promote scientific research, particularly among the students who will be our future leaders.

DESDE EL ESCRITORIO DE LA EDITORA
DRA. LIZBETH ROMERO-PÉREZ

Hoy, que experimentamos la pandemia causada por el Covid-19 debemos tener presente cuán importante es promover la investigación en la educación y que nuestros estudiantes se preparen en el campo de las ciencias. Es por ello que con gran orgullo deseamos presentar nuestro séptimo volumen de *Inter Scientific*. En este podrán encontrar dos artículos de investigación y un artículo de revisión, al igual que resúmenes de otras investigaciones que se han realizado en nuestro Recinto durante este pasado año académico.

La primera investigación nos lleva a explorar el comportamiento alimenticio de los colibríes y cómo el urbanismo les afecta. Este trabajo fue realizado por estudiantes de la Universidad de Puerto Rico, Recinto de Mayaguez bajo la dirección del Dr. Alberto Puente Rolón. El segundo trabajo de investigación considera metodologías moleculares para identificar adecuadamente variedades del mangó. Dicho trabajo se basó en extracción de ADN de hojas de árbol de mangó colectadas en el área norte de Puerto Rico. Fue realizado por estudiantes de nuestro Recinto del programa de Biotecnología. También encontrará un artículo de revisión sobre el virus Epstein Barr y enfermedades asociadas. Este escrito fue preparado por estudiantes de Medicina de la Universidad Autónoma de Guadalajara en México, bajo la mentoría de la Dra. Olivia Torres-Bugarín. En la sección *La Investigación en el Campus*, encontrará resúmenes de otras investigaciones que se han estado realizando en los campos de farmacología-bioquímica y ecología.

Aprovecho la ocasión para felicitar a todos los estudiantes y sus profesores por el excelente trabajo realizado durante el pasado año académico. También deseo agradecer a todo el personal que ha apoyado esta iniciativa incluyendo a nuestro Rector el Dr. Rafael Ramírez Rivera, la Dra. Karen Woolcock, Decana de Asuntos Académicos, Dra. Wanda Balseiro, Decana Asociada de Asuntos Académicos y gestora del origen de este proyecto, a toda la facultad, técnicos de laboratorio, personal administrativo y estudiantes.

FROM THE EDITOR'S DESK
DR. LIZBETH ROMERO-PÉREZ

As we experience the pandemic caused by Covid-19, more than ever, we must be aware of the importance of promoting research in education and that students complete their studies in science. That is why we proudly present to you our Seventh Volume of *Inter Scientific*. In this occasion, you will find two research and one review articles as well as abstracts of other research done at our Campus during the past academic year.

The first research project takes us to explore the foraging behavior of the Antillean Mango and how it is affected by urbanization. Students of the University of Puerto Rico in Mayaguez, under the direction of Dr. Alberto Puente Rolón, did that investigation. The second project evaluated molecular methods for the proper identification of mango varieties. DNA from mango tree leaves collected in the northern area of Puerto Rico was isolated. Students of the Biotechnology Program at our Campus developed the project. In addition, you will find a review article on Epstein-Barr virus and associated diseases. Students of the School of Medicine at Universidad Autónoma de Guadalajara in México under the mentorship of Dr. Olivia Torres-Bugarín prepared the manuscript. In the section *Research on Campus* you will find abstracts of other research projects being worked in the fields of pharmacology-biochemistry and ecology.

I take this opportunity to congratulate all the students and their professors for the excellent work. Also, want to thank our chancellor, Dr. Rafael Ramírez Rivera, Dr. Karen Woolcock, Dean of Academic Affairs, Dr. Wanda Balseiro, Associate Dean of Academic Affairs and promoter of this project, the faculty, laboratory technicians, administrative personnel and students for their continuous support.

Foraging behavior of the Antillean Mango *Anthracothorax dominicus* in urban environments

Comportamiento de forrajeo del zumbador dorado *Anthracothorax dominicus* en ambientes urbanos

Talavera-Arboniés, M.L, García-Cabrera, S.M, and Puente-Rolón, A.R

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ABSTRACT

Hummingbirds are characterized by three main feeding strategies: trapliners, territorialists or generalists. However, these strategies can be impacted by the rampant urbanization that results in change of resource availability and habitat. Hummingbirds in Puerto Rico have been classified as trapliners yet have not been studied since 1984. This study focused on analyzing the feeding strategies and behaviors adopted by the Antillean Mango (AM) *Anthracothorax dominicus* on urban environments. We identified their flower preferences and examined the flowers' nectar quantity and quality. We also made focal observations to determine the foraging behavior of the individuals. We discovered that they still employed trapliner strategies and that nectar reward and structure play a major role in determining the amount of time spent feeding. We found that higher nectar rewards resulted in lower feeding times. Also, we found that after a habitat disturbance the AM changes its foraging behavior to deal with the change.

RESUMEN

Los colibríes están caracterizados por tres tipos de estrategias alimenticias: "trapliners", territorialistas o generalistas. Sin embargo, la urbanización podría impactarlas por cambios en disponibilidad de recursos y hábitat. Los colibríes de Puerto Rico se clasifican como "trapliners", pero no se estudian desde el 1984. Este estudio se enfoca en el análisis de estrategias y comportamientos alimenticios empleados por el Zumbador Dorado (ZD) (*Anthracothorax Dominicus*) en ambientes urbanos. Se identificaron preferencias de flores y se examinó su cantidad y calidad de néctar. También, se realizaron observaciones focales, determinando el comportamiento de forrajeo de los individuos. Descubrimos como todavía emplean la estrategia de "trapliner" y cómo la recompensa de néctar y estructura de flor juegan un papel esencial en determinar tiempo alimentándose. Se encontró que recompensas mayores del néctar resultaron en menos tiempo forrajeando. También se observó como después de disturbios de hábitat el ZD cambia su comportamiento de forrajeo para lidiar con este.

KEYWORDS Traplining, nectar, urban disturbance, island

PALABRAS CLAVE Traplining, néctar, disturbios urbanos, isla

INTRODUCTION

Across many island ecosystems, hummingbirds serve a vital role in the pollination of plants. Many of them have mutualistic relationships exclusive between them, thus highlighting their importance for study (Dalsgaard et al., 2018). Hummingbirds differ in feeding strategies throughout various ecosystems depending on food availability and resource distribution. Hummingbirds are characterized by three main feeding strategies: either trapliners, territorialists or generalists. (Feininger & Colwell, 1978). While trapliners follow set feeding routes with patches of food spread throughout the route, territorialists adamantly defend flower resources throughout the day from other individuals. Generalists in turn feed on dispersed flowers and clumps without adhering to the previous strategies. Puerto Rico is home to five species of hummingbird (*Anthracothorax dominicus, Chlorostilbon maugaeus, Eulampis holosericeus, Anthracothorax viridis, Orthorhyncus cristatus)* and all of them are known to employ the trapliner strategy (Kodric-Brown et al., 1984). Yet one of the many obstacles facing these organisms is the increase in rampant urbanization.

The development of urbanized areas leads to a loss of natural habitat, the addition of impervious surfaces, an increase in local temperatures, and a shift in the availability of food sources. (Zuzula, 2017). Puerto Rico is an island that has observed heavy

urbanization in recent years (Martinuzzi et al., 2006), yet its impact on hummingbirds has not been properly studied. The last study done on the island achieved an in depth understanding of the feeding habits and behavior of the five species in natural ecosystems. Since 1984 no study has analyzed the behavior of these animals and no study has been performed in urban environments. This paper then analyzed how these animals adapt to urban-rural environments with plant species foreign to their natural habitat. All species of hummingbird observed were recorded in two sites. The first study site was at the Serpentinata at the University of Puerto Rico Mayaguez campus and the second at Calle Monte del Estado in Mayaguez. Both sites exhibited cultivated exotic flower species; *Heliconia psittacorum* and *Ixora coccinea* respectively. Analysis was only possible with *Anthracothorax dominicus* due to lack of presence from other species. In this syudy, we describe the feeding habits and overall behavior of *A. dominicus* in urban environments and discuss the underlying reasons for such behavior.

MATERIALS AND METHODS

Study sites

There are no previous studies, which specify the location of the AM in urban areas. Therefore, we conducted a series of observations throughout the urban areas of Mayaguez, Puerto Rico. These observations consisted on the identification of potential flowers that hummingbirds could feed on. Once these flowers were identified, observers did focal observations on the flowers at 6:00-8:00 and at 16:00-18:00. If one hummingbird was spotted, focal observations were repeated twice to make sure the location was frequented and could be used as a study site. We also used recommendations from photographers and a bird spotting website (*Ebird.com*) to identify other potential study sites.

Two principal study sites were found. The first site was at the Serpentinata at the University of Puerto Rico Mayaguez (UPRM) campus. On this site we found a female AM who frequented the *I. coccinea* found at the garden that surrounds the student center. The second was at Calle Monte del Estado, also in Mayaguez. Here, we found a spot of *H. psittacorum* that was frequented by both an adult and a juvenile male AM. We also found other sites that were visited by a female and a male, but they were not as frequented as the sites mentioned above. However, they were used for this study because the flowers used by the female at the campus were cut. Therefore, we looked for other spots inside the campus to see the behavior of the female after a disturbance.

Foraging observations

One of the main objectives of this study was to learn about the foraging behavior of the AM. Thus, we made a series of standardized focal observations. Each observation was made during a 15-minute time lapse. During this time, we wrote every activity that the hummingbirds did and the time in seconds that they spent performing the activity. There were two main activities: foraging and perching. Foraging behavior referred to every activity that was related to eating such as flying to and around the flowers and extracting nectar from them. Perching behavior was recorded when the hummingbird perched on a branch to rest between foraging flights or to groom.

Flower preference

We identified every flower visited by the AM in urban areas. After identifying such flowers, we collected data about their nectar reward. We used capillary tubes (I.D. 1.1-1.2mm, 5µL) to extract the nectar from the flowers and determine the volume that is available for the hummingbirds at the morning. Then, we placed the sample of nectar in a portable refractometer. The refractometer measures the percentage of brix in the nectar. Brix is used to identify the amount of dissolved sugar in a solution. Therefore, we were able to determine the quality of the sample of nectar.

Statistical analysis

To determine if there were significant differences between the foraging and perching behavior of the male and female, we conducted a t-tests on both activity variables. On the other hand, we used non-parametric ANOVA to determine if statistical differences existed between the brix % of the flowers and their volume.

RESULTS

Foraging observations

Observations (113) of 15 min were made in different locations: 51 in the neighborhood, 32 in campus and the last 30 were distributed around other locations at the campus besides the principal one. We were able to record foraging behaviors of 3 individuals. Two were males, one adult and one juvenile. They both were located at the neighborhood. During the 51 observations made at that study site, we saw only two encounters between both. These resulted in a chase away from the flower resource. The other individual was the female and she was located at the garden that surrounds the student center at the campus. It is important to note that during the observations of the female on that spot, the flowers that the female foraged were cut down due to security protocols. This resulted on a division of observations before and after the disturbance. We were able to conduct 16 replicas before the disturbance and 16 observations after it. Therefore, we were able to see changes on the foraging behavior of a female AM.

Observations of the adult and juvenile male were grouped in one category: male. Then, we compared it to the behavior recorded for the female before the disturbance. Depending on the time of observation on each site, we determined the percentage of time that

individuals spend. The percentage time male spend was 9.34% whereas female was present 31.33% of the time before the disturbance. Once the disturbance happened, she was only present 8.10% of the time.

When the hummingbirds arrived at the feeding spot, they engaged in a repetition of activities. They spent a certain amount of time foraging the flowers and then switched to perch on a tree branch near them. On average, they spent more time perching than foraging. Figure 1 shows the average time that the hummingbirds spent when they chose to perform one of the two activities. On the other hand, Figure 2 shows the amount of time that each sex spent foraging daily. This result is the sum of the seconds spent on every foraging visit per day. On average, females spent more time foraging than males.

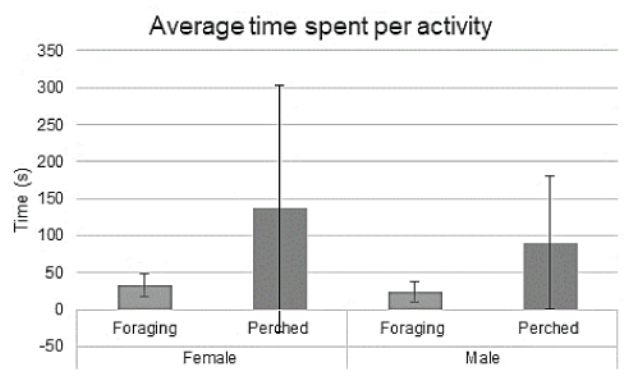

Figure 1. This figure shows the average amount of time (in seconds) that the individuals engaged per activity. While perching differences between males and females were not statistically significant (p=0.09), foraging differences we significant (p=0.02). Bar lines represent the standard deviation.

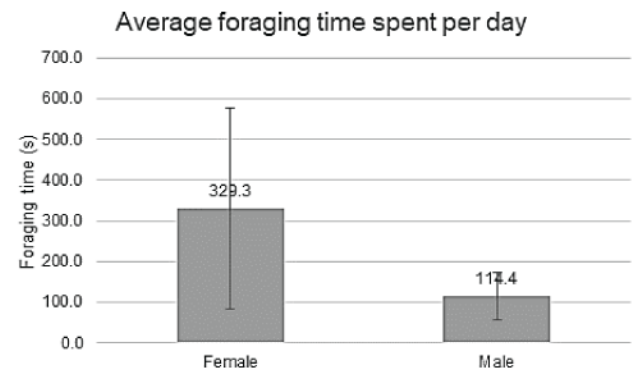

Figure 2. This figure shows the average foraging time (in seconds) spent per day. The differences between female and male were statistically significant (p=0.04). Bar lines, represent the standard deviation.

We already saw that the female decreased her percentage of time after the disturbance happened. However, she fed from the last bunch of flowers available on one day before their removal. If we sum the total time spent foraging the flowers before disturbance and compare it to after, she also decreases the time of foraging (Figure 3).

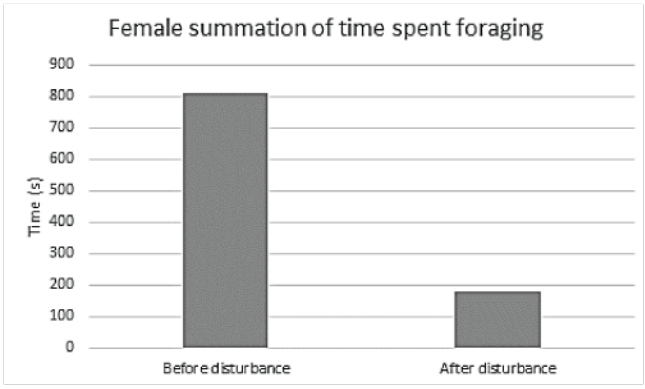

Figure 3. This figure shows the sum of time (in seconds) that the female spent foraging on the site before and after the disturbance.

Flower preference

As mentioned before, the diversity of flowers present at urban areas is very different from the forest. The flowers that Kodric-Brown (1984) identified as resource to the AM, are not present in urban areas. We could identify five flowers that the AM visited at our urban study sites. The species' name are the following: *Ixora coccinea* (cruz de malta), *Heliconia psittacorum*, *Billbergia pyramidalis* (bromelia), *Alpinia zerumbet* (shell ginger), and *Cordia sebestrana* (vomitel). *I. coccinea, B. pyramidalis and A. zerumbet* were found at the UPR campus, being *I. coccinea* the most frequented by the female. *H. psittacorum* was found at the neighborhood and visited by the males. Last, the *C. sebestrana* was found at the Suau Park close to the UPR and was visited twice by a male. After extracting the available nectar, we determined its quantity (volume) and quality (brix %) and compared it between the species as Figures 4-5 show. We were not able to extract the nectar from the *B. pyramidalis* because the sighting of visitation to the flower was made on preliminary observations. *H. psittacorum* had both more quantity and better quality of nectar than *I. coccinea.* Finally, we tested the relationship between the quality of the nectar with the percentage of presence as shown in Figure 6. This shows that higher nectar quality results in lower presences percentage.

DISCUSSION

Consistent with previously described feeding behaviours, the AM studied exemplified trapliner strategies for all individuals. Based on the repeated periods of feeding followed by flying away from site only to return minutes later, a trapliner strategy with a repeated route is probable. Although one example of territorialism was

observed between the juvenile and the adult male, it was not repeated sufficient to merit a change in feeding strategy. This leads us to believe that although the AM exemplified trainer strategies, at the time of encountering a rival hummingbird in the same resource the dominant individual will chase out the rival. Both male and female AM shared no significant difference in perching time despite uneven distribution of foraging time. Perching requires varied amounts of rest depending on activity levels so regardless of different flower sources rest varies significantly for both.

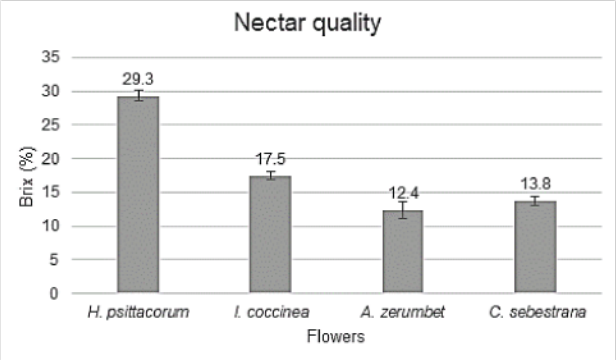

Figure 4. This figure represents the quality of the nectar of the different flowers visited by the AM. The differences were all statistically significant ($p<0.0001$). Bar lines represent the standard deviation.

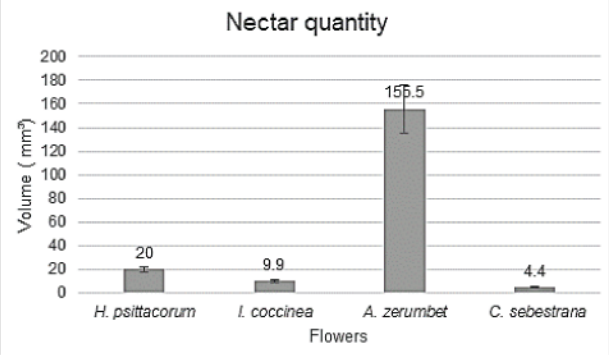

Figure 5. This figure shows the quantity of the nectar of the different flowers visited by the AM. Their differences were all statistically significant ($p<0.0001$). Bar lines represent the standard deviation.

The difference in foraging activity can be explained by lower nectar quality and quantity observed in *I. coccinea* making the female compensate for the decreased nectar quality available compared to *H. psittacorum*. This is consistent with Kodric-Brown and colleagues (1984) observation that "The small quantity of nectar secreted by short-tubed flowers is below the threshold usually required for economical foraging by large hummingbirds". The *I. coccinea* is a short-tubed flower hence explaining why the female needs more visits compared to the males. When comparing total time spent per day feeding we can also see a stark difference between females and males whereas the female spent much more time than the males feeding. Again explained by the difference in quantity and quality of nectar in *H psittacorum*. The males must spend less time feeding overall because of the huge energy reward given by the Heliconia and the female needs more visits to reach its required energy input.

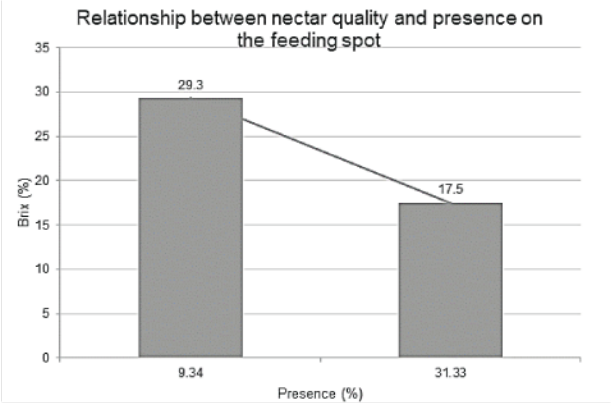

Figure 6. This figure shows the inverse relationship between nectar quality (Brix %) and the percentage of presence.

The standard deviation observed in both feeding times can be accounted by the feeding strategy that they employ. By being trapliners, clusters of flowers are scattered in the route and varying nectar levels in clusters may influence time spent in the study sites. in the female's case considering the little nectar offered by the ixora she may have had to travel more compared to the males where once again quantity and quality of nectar influences the variability of feeding times in the males favor. Upon taking into account all of the data we can observe an inverse relationship between an increase in Brix value and a decrease in overall presence as explained by how higher energy rewards merit less time spent consuming flowers compared to lower Brix valued flowers. At first the goal of the UPRM study site was merely observing the female in the garden yet near the end of the study the site experienced varied cuts to the ixora coccinea leading to a direct observation to the animals response to an urban disturbance. Compared to before the disturbance the female's total foraging time plummeted. Considering the gradual cut of flowers from the site, its apparent how the female reduces time foraging in accordance with flowers available. Hence the complete lack of presence near the end of the study where the individual virtually abandoned the site from its route upon not having sufficient flowers to sustain it. We can infer then that traplining hummingbirds modify their routes in accordance with available resources and can change them concurrently with environmental change.

CONCLUSION

AM in the Mayaguez area maintain a trapliner feeding strategy as

established by Kodric-Brown et al, in 1984. All species of hummingbird on the island engage in the strategy and it is still relevant in urban environments where concentrated resources are possibly even more spread out than natural counterparts, thus requiring distinct routes to maximize energy input. Moreover, trapliners in urban environments are at the mercy of cultivated flowers possibly requiring more scrutiny when deciding routes compared to natural counterparts. When visiting said routes there is an inverse relationship where higher Brix valued flowers show a decrease in time spent feeding when compared to lower Brix valued flowers. This is due to the energy offered by the flowers being sufficient to merit less time feeding on them. Hence hummingbirds will spend more time feeding on flowers if they offer less nutritional value.

Urbanization is characterized by changing environments, such as observed in the UPRM study site. Here the consequences of disturbing food resources for the female led to a direct decrease in time spent in area until inevitably leaving the site completely. Highlighting the importance of how cultivated flowers, though exotic, can still prove useful for the AM and how we can minimize the impact of urban environments by having suitable resources in place.

REFERENCES

Dalsgaard, B., Kennedy, J. D., Simmons, B. I., Baquero, A. C., González, A. M. M., Timmermann, A., … Rahbek, C. (2018). Trait evolution, resource specialization and vulnerability to plant extinctions among Antillean hummingbirds. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1875), 20172754. doi: 10.1098/rspb.2017.2754.

Feinsinger, P., & Colwell, R. K. (1978). Community Organization Among Neotropical Nectar-Feeding Birds. *American Zoologist*, *18*(4), 779–795.

Kodric-Brown, A., Brown, J., Byers, G., & Gori, D. (1984). Organization of a Tropical Island Community of Hummingbirds and Flowers. *Ecology, 65*(5), 1358-1368. doi:10.2307/1939116.

Martinuzzi, S., Gould, W. A., & Ramos-González, O. M. (2007). Land development, land use, and urban sprawl in Puerto Rico integrating remote sensing and population census data. *Landscape and Urban Planning 79*, 288–297.

Zuzula, C. C. (2017). Effects of urbanization on the relative abundance of hummingbirds (*Archilochus alexandri and Archilochus colubris*) as measured by resource removal rates. *Graduate Council of Texas State University*, 1–34.

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Evaluation of different protocols to isolate DNA from mature mango (*Mangifera indica*) tree leaves for the detection of polymorphism using Single Sequence Repeat (SSR) markers and Randomly Amplified Polymorphic DNA (RAPD)

Evaluación de diferentes protocolos para aislar ADN de las hojas maduras de los árboles de mango (*Mangifera indica)* para la detección de polimorfismo utilizando microsatélites ("Single Sequence Repeat" (SSR)) yAmplificación Aleatoria de ADN Polimórfico ("Randomly Amplified Polymorphic DNA" (RAPD))

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ABSTRACT

***Mangifera indica* (mango) is known as the king of the fruits for its economic importance and there are more than 1,000 varieties. Identification of mango is complicated since morphological traits are influenced by climate. To ensure proper identification, molecular markers like Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) can be employed. In order to achieve this, DNA of good quality must be isolated. Mango contains high concentration of phenols and other compounds that interfere with DNA isolation. Previous studies show that isolating DNA from the leaves of the tree is feasible, but it requires many cleaning steps. The objective of this research is to evaluate five different protocols to isolate DNA that can be used for PCR applications. Our results show that quality DNA was isolated using two of the tested protocols. Four SSR markers tested and the RAPD analysis detected low levels of polymorphism. More samples and molecular markers should be tested in order to establish a profile for the mango varieties in the northern area of Puerto Rico.**

RESUMEN

Mangifera indica (mangó) es conocido como el rey de los frutos debido a su importancia económica y se han identificado más de 1,000 variedades. El proceso de identificación del mangó es complicado ya que los rasgos morfológicos varían con el clima. Para garantizar identificación adecuada se pueden utilizar técnicas moleculares como la Amplificación Aleatoria de ADN Polimórfico (Randomly Amplified Polymorphic DNA (RAPD)) y microsatélites (Simple Sequence Repeats (SSR)). Para poder emplear estos métodos se requiere ADN de alta calidad. *M. indica* contiene alta concentración de fenoles y otros compuestos que interfieren con el aislamiento de ADN. Estudios anteriores muestran que aislar el ADN de las hojas del árbol del mangó es posible, pero requiere muchos pasos de limpieza. El objetivo de esta investigación es evaluar cinco protocolos diferentes para aislar ADN que pueda utilizarse para aplicaciones moleculares. Nuestros resultados muestran que ADN de alta calidad fue aislado utilizando dos de los protocolos evaluados. Los cuatro marcadores SSR y el análisis RAPD detectaron niveles bajos de polimorfismo. Se deben analizar más muestras y marcadores moleculares para establecer un perfil de las variedades encontradas en la zona norte de Puerto Rico.

KEYWORDS Mango, DNA isolation, SSR, RAPD

PALABRAS CLAVE Mango, Aislamiento de ADN, SSR, RAPD

INTRODUCTION

Mango (*Mangifera indica)*, constitutes 5.5% of all fruits and 50% of the tropical fruits sold in the world (Viruel et al., 2005). For that reason it is commonly known as the King of the Fruits (Krishna & Singh, 2007). Currently there over 1,000 varieties of mango in the world (Mango, 2012). Identifiyng mango is a challenge since morphological traits are affected by climate. The problem exacerbates with commercial crops (Azmat et al., 2016, Uddin & Cheng, 2015; Viruel et al., 2005). With the advent of molecular techniques during the last decades, identification has improved. Molecular markers allow precise identification through the detection of

polymorphisms (Sharma et al., 2010). Molecular markers also provide improvement in breeding programs by allowing the breeder to visualize the genetic diversity in their crops and selecting the most stable mango trees for commercial harvest. It is important to mention that even though in the last decade more genetic studies have been done,, information of mango genetics is scarse (Uddin & Cheng, 2015).

Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) are molecular markers commonly used for plant analysis. RAPD is a molecular technique that uses random short sequence primers for Polymerase Chain Reaction (PCR). Those primers allow DNA amplification that can be visualized as bands with different molecular weights that establish a pattern. The band patterns are used for the identification of polymorphism (Sharma et al., 2010). In 2011, Souza and colleagues (Souza et al., 2011) used RAPD markers for the identification of genetic differences and similarities in different varieties of Brazilian mangoes.

Single sequence repeat (SSR) analysis uses PCR and specific primers for the amplification of microsatellites. Microsatellites are short tandem repeats composed of 1-6 nucleotides. When a sample is amplified, the molecular weight depends on the number of repeats of the nucleotides in that allele. Azmat and colleagues (Azmat et al., 2016) used SSR for the analysis of mango crops. They tested 101 primers in 48 commercial crops. Twenty-nine of the 101 SSR primers were efficient in the detection of polymorphism.

Molecular markers are great tools for the identification of mango, but requiere the isolation of DNA of good quality. Mango as well as other plants has high concentration of phenolic compounds, polysaccharides, proteins and other metabolites. Those compounds make it difficult to be able to isolate pure DNA. Phenolics can bind to the DNA and form a brown precipitate making it difficult to use for molecular applications. Several methods have been tested for the isolation of DNA from mango (Azmat et al., 2012; Uddin et al., 2014; Healey et al., 2014). The most efficient method relies on the isolation of DNA from mango tree leaves, preferably, mature mango leaves. Even thou they have a higher concentration of biochemical compounds, they are more associable through the year in comparison with young leaves (Azmat et al., 2012; Uddin et al., 2014). In this study, we want to evaluate different protocols to isolate DNA from mango tree leaves for the detection of polymorphisms using Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers.

MATERIALS AND METHODS

Samples

Twelve (12) samples from eleven different mango trees were collected in the northern area of Puerto Rico (Table 1). They were collected in "Ziploc" bags and stored at 4 °C. After arrival, leaves were washed by rubbing with paper and water. Then the leaves were rinsed three times for 3 minutes in distilled water (shaking). Leaves were dried and stored at -80 °C.

Three (3) leaves of each sample were used. Secondary stem of each leave was removed using a scalpel. Samples were macerated using liquid nitrogen and mortar and piston previously frozen at -40 °C. Material was weighed and stored in microtubes at -80 °C.

Table 1. Coordinates of the collection sites. Leaves from eleven different trees located in the northern area of Puerto Rico were collected.

Sample	Coordinates
1	18°26'40" N, 66°38'19" W
2	18°26'40" N, 66°38'20" W
3	18°27'14" N, 66°35'49" W
4	18°27'39" N, 66°35'56"W
5	18°26'34" N, 66°39'03"W
6	18°26'39" N, 66°39'05"W
7	18°26'45" N, 66°40'03"W
8	18°26'46" N, 66°40'14"W
9	18°26'46" N, 66°40'04"W
10a 10b	18°27'27" N, 66°36'38"W
11	18°29'07" N, 66°47'09"W

DNA extraction

Five methods of DNA extraction were evaluated: three organic methods as described in Uddin et al., 2014, Healey et al., 2014 and Rodrigues et al., 2007; and two commercial kits from the company Qiagen. For all the organic methods β-mercaptoethanol (BME) in the buffers was added when ready to use.

Uddin et al., 2014

Briefly described, 0.3 g of macerated leaves were used. For tissue disruption, solution 1 (0.4 M glucose, 20 mM ethylenediaminetetraacetic acid EDTA pH 8, 3% polyvinylpyrrolidone (PVP) -40, 0.2% BME) and pre-heated solution 2 (2% CTAB Cetyl Trimethyl Ammonium Bromide, 1.4 M NaCl, 100 mM Tris pH 8 , 20 mM EDTA pH 8 and 0.15% BME) were used. Proteins were extracted using chloroform: isoamyl alcohol 24:1. This step was repeated 3 times or until a clear supernatant was obtained. DNA was precipitated using 100% ethanol and sodium acetate followed by rinse with 70% ethanol. Pellet was dried, resuspended with 1X TE and stored at -20 °C.

Healey et al., 2014

Briefly, tissue (0.250 g) was disrupted using pre-heated extraction in buffer (100 mM Tris pH 7.5, 25 mM EDTA, 1.5 M NaCl, 2%

CTAB, 0.3% of β-mercaptoethanol) and incubating at 65 ºC. Protein extraction was done using chloroform: isoamyl alcohol followed by treatment with RNase A (10 mg/ml). DNA was precipitated and rinsed with 5M NaCl, 95% cold ethanol and 70% ethanol respectively. Pellet wad dried, resuspended with TE buffer and stored at -20 ºC. Twelve samples were analyzed in duplicate.

Rodrigues et al., 2007

This method was developed for RNA extraction. Modifications are described. For this method, 100 mg of previously macerated mango leaf were used. Tissue was disrupted using homogenization buffer (0.2 M boric acid, 10 mM EDTA pH 7.6, 10 mM Tris pH 7.5, 0.5% SDS and 0.286 M of β-mercaptoethanol). Extraction was done using chloroform isoamyl alcohol and CTAB solution (2% CTAB and 3 M NaCl). DNA was precipitated using cold isopropanol and sodium acetate followed by rinsing with 70% ethanol. The pellet was dried and resuspended with TE Buffer.

DNeasy Plant Mini Kit and DNeasy Power Plant Pro Kit

Both kits from Qiagen (DNeasy Plan Mini Kit and DNeasy Power Plant Pro Kit) were used following manufacturers instructions.

DNA Analysis

The quality and concentration of the DNA was determined using spectrophotometry. In addition, all samples were analyzed by eletrophoresis in 1% agarose gels.

Randomly Amplified Polymorphic DNA (RAPD)

For RAPD reaction, 15 ng of DNA and Go Taq Green Master Mix was used. The primers used were A01, A09, G03 and N05 (Souza et al., 2011) (Table 2). The total reaction volume was 20 µl. Reaction included and initial denaturalization step of 1min at 92 °C, followed by 40 cycles of amplification (denaturalization 1 min at 92 °C, annealing 1 min at 35 °C and extension for 2 minutes at 72 °C) and a final extension of 5 min at 72 °C. Samples were analyzed by 1.4% - 2% agarose gel electrophoresis.

Table 2. Sequence of primers used for RAPD PCR.

Primer	Sequence
A01	CAG GCC CTT C
A09	GGG TAA CGC C
G03	GAG CCC TCC A
N05	ACT GAA CGC C

Single Sequence Repeat (SSR) markers

For SSR analysis, 30 ng of DNA and Go Taq Green Master Mix was used. The primers used are described in Table 3 (Azmat et al., 2016). The total volume of the reaction was 25 µl. Reaction included and initial denaturalization step of 5 min at 94 °C, followed by 30 cycles (denaturalization 30 sec at 94 °C, annealing 30 sec at 46-57 °C and 1 minute extension at 72 °C) and a final extension of 10 min at 72 °C. Samples were analyzed by 2.5% agarose gel electrophoresis.

Table 3. Sequence of primers used for SSR analysis and their annealing temperature.

Primer	Sequence (5'-3')	A (ºc)
mMiCIR005 FWD	GCC CTT GCA TAA GTT C	51
mMiCIR005 RV	TAA GTG ATG CTG CTG GT	51
mMiCIR016 FWD	TAG CTG TTT TGG CCT T	51
mMiCIR016 RV	ATG TGG TTT GTT GCT TC	51
mMiCIR027 FWD	ACG GTT TGA AGG TTT TAC	51
mMiCIR027 RV	ATC CAA GTT TCC TAC TCC T	51
MiIIHR20a FWD	CCT AAC GCG CAA GAA ACA TA	55
MiIIHR20a RV	ACC CAC CTT CCC AAT CTT TT	55

Samples were analyzed for molecular weight determination using CLIQS software/ 1D gel. For dendrogram generation, samples were analyzed by the UPGMA method using PyElph 1.4 software.

RESULTS

We were able to isolated gDNA using four of the five methods. Figure 1 shows an example of gDNA extracted using the commercial kit DNeasy Plant Mini kit from Qiagen.

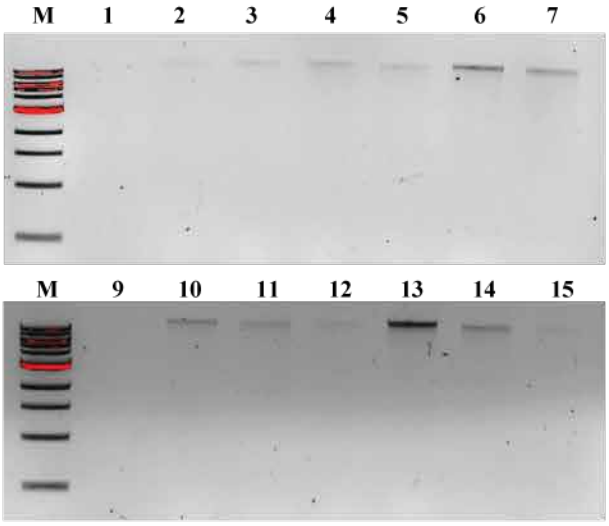

Figure 1. Agarose gel electrophoresis (1%). gDNA extracted from mango tree leaves using DNeasy Plant Mini Kit (Qiagen). Lane M (1 Kb marker), lanes 1 and 9 (empty), lanes 2-7 and 10-15 (samples).

Tables 4 to 7 show the quality and concentration of the DNA we were able to extract using four of the five methods tested. Results show extraction of DNA from the twelve samples collected and analyzed in duplicate. For the protocol adapted from Rodrígues et al., 2007 only 6 samples were analyzed since the purity ratio of the DNA extracted was too high (2.0-3.3, data not shown).

Table 4. Concentration and purity of gDNA extracted using the protocol described in Uddin et al., 2014.

Sample	Concentration (µg/ml)	Abs 260/280
1	0.9/0.1	6.5/1.0
2	1.95/2.39	1.1/1.6
3	2.52/3.83	7.6/6.6
4	4.35/88.75	1.1/1.9
5	1.4/1.1	2.7/2.9
6	8.2/0.2	2.0/0.3
7	21.0/0.8	1.2/0.8
8	0.4/0.1	n/a
9	10.8/4.4	1.8/3.1
10a	1,227.5/1,247.4	2.0/2.1
10b	3.6/2.9	3.8/4.6
11	2.5/3.9	6.1/3.8

Table 5. Concentration and purity of gDNA extracted using the protocol described in Healey et al., 2014.

Sample	Concentration (µg/ml)	Abs 260/280
1	37.2/25.0	2.1/2.0
2	48.2/34.0	2.2/2.0
3	2194.8/160.0	1.9/2.0
4	110.2/134.1	3.1/2.9
5	36.2/29.8	2.0/2.1
6	19.0/22.9	1.9/1.9
7	31.4/32.4	1.9/1.9
8	16.8/9.2	2.1/2.2
9	38.8/12.6	2.1/1.6
10a	47.7/345.4	1.8/2.0
10b	37.2/68.0	1.8/1.9
11	14.5/26.0	2.0/1.8

Table 6. Concentration and purity of gDNA extracted using the DNeasy Plant Mini kit (Qiagen).

Sample	Concentration (µg/ml)	Abs 260/280
1	3.5/5.8	3.2/1.9
2	6.7/7.1	1.9/1.9
3	8.0/6.6	1.7/2.1
4	6/4/5.0	4.6/4.4
5	6.6/6.5	1.7/1.8
6	4.0/4.8	2.0/1.8
7	5.6/4.6	1.5/2.1
8	2.5/4.6	2.8/1.6
9	3.4/3.1	1.5/1.6
10a	7.6/6.9	1.6/1.5
10b	5.7/6.2	1.4/1.4
11	4.6/4.5	1.2/1.5

Table 7. Concentration and purity of gDNA extracted using the DNeasy Power Plant Pro kit (Qiagen).

Sample	Concentration (µg/ml)	Abs 260/280
1	9.9/9.4	1.3/1.0
2	54.0/46.8	1.5/1.4
3	18.7/13.8	1.1/0.9
4	13.0/9.0	1.2/1.4
5	22.3/36.1	1.7/1.6
6	5.7/7.2	0.8/0.9
7	5.8/1.2	0.7/0.2
8	42.7/31.8	1.6/1.3
9	12.2/7.6	1.3/1.4
10a	15.8/8.0	1.1/1.5
10b	14.9/25.4	0.9/1.2
11	7.2/8.4	1.6/1.8

For the molecular marker analysis we used the DNA extracted with the DNeasy Plant Mini Kit and the method described in Healy et al., 2014.

Figures 2 and 3 show the RAPD results and figures 4 to 7 show the results for the SSR analysis.

DISCUSSION

The objectives of this study were to evaluate and optimize a protocol to isolate DNA from mature mango tree leaves. Also, to detect polymorphisms in mango using Randomly amplified poly-

morphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers.

DNA extraction

Five different protocols were tested; three organic methods published by Uddin (Uddin et al., 2014), Healey (Healey et al., 2014) and Rodrigues (Rodrigues et al., 2007) and two commercial kits from the company Qiagen DNeasy Plant Mini kit and DNeasy Power Plant Pro kit.

The protocol described by Uddin et al., 2014, allowed isolation of DNA but at low concentrations ranging from 0.2 to 10.8 µg/ml in most samples (3 samples have higher concentrations 21, 89, 1,247 µg/ml) and a low degree of purity (Table 3). These results may be a consequence of the many steps and time required for the protocol. Of all the methods it was the longest ranging from 14 to 36 hours.

The protocol described by Healey et al., 2014, was easier to perform in comparison with the Uddin et al., 2014. This protocol allowed the isolation of high concentrations of DNA ranging from 14.5 to 160.0 µg/ml (2 samples had higher concentrations 345 and 2194 µg/ml). In addition, the range of purity of the isolated DNA was acceptable ranging from 1.8 to 2.1 in the majority of the cases (Table 5). The DNA was of enough quality for RAPD reaction (Figure 2).

The protocol described by Rodrigues et al., 2007 was a protocol designed to isolate RNA. After a preliminary test with six samples and various modifications, the extraction resulted with high levels of RNA as expected for the protocol. No futher test was performed using this method.

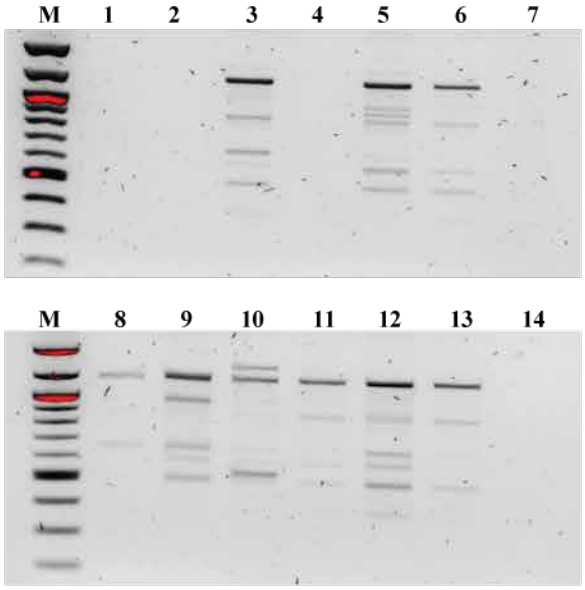

Figure 2. Agarose gel electrophoresis (2%). RAPD pattern using A01 primer. M (marker 100 bp), lane 1 (empty), lanes 2-13 (samples), lane 14 (negative control).

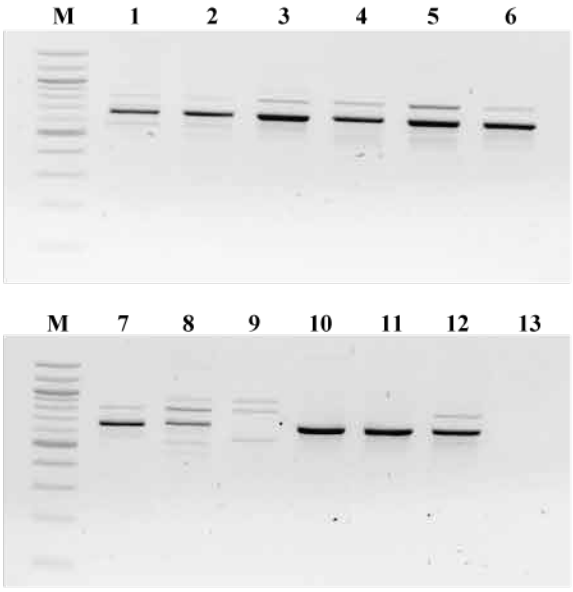

Figure 3. Agarose gel electrophoresis (2%). RAPD pattern using G03 primer. M (marker 100 bp), lanes 1-12 (samples), lane 13 (negative control).

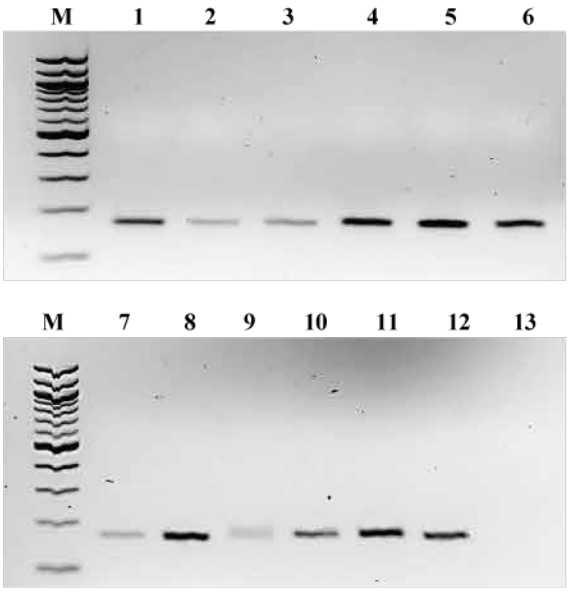

Figure 4. Agarose gel electrophoresis (2.5%). SSR-PCR using mMiCIR005 primer set. M (marker 100 bp), lanes 1-12 (samples), lane 13 (negative control).

The two commercial kits evaluated, were easy to perform and fast. The drawback was that the DNA concentrations were low. With the DNeasy Mini kit the concentration of isolated DNA ranged

from 3.4 to 8.0 µg/mL (Table 6). Our results were consistent with a previous study performed by Sharma et al., 2014. They also observed low concentrations of DNA. Besides the low concentration detected by spectrophotometry, when performing the agarose gel electrophoresis, a sharp band of gDNA was observed for all samples (Figure 1). In addition, the quality was enough for RAPD and SSR reactions (Figure 3-7). With the DNeasy Power Plant Pro kit DNA concentration was from 1.2 to 54 µg/mL. The purity was 0.20-1.8 (Table 7).

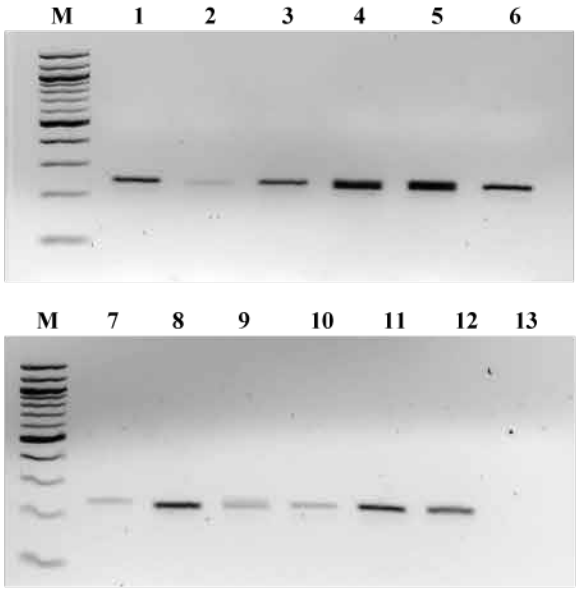

Figure 5. Agarose gel electrophoresis (2.5%). SSR-PCR using mMiCIR016 primer set. M (marker 100 bp), lanes 1-12 (samples), lane 13 (negative control).

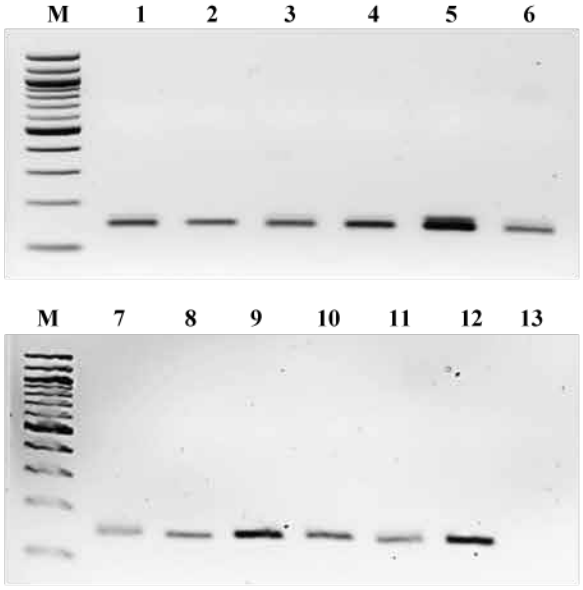

Figure 6. Agarose gel electrophoresis (2.5%). SSR-PCR using mMiCIR027 primer set. M (marker 100 bp), lanes 1-12 (samples), lane 13 (negative control).

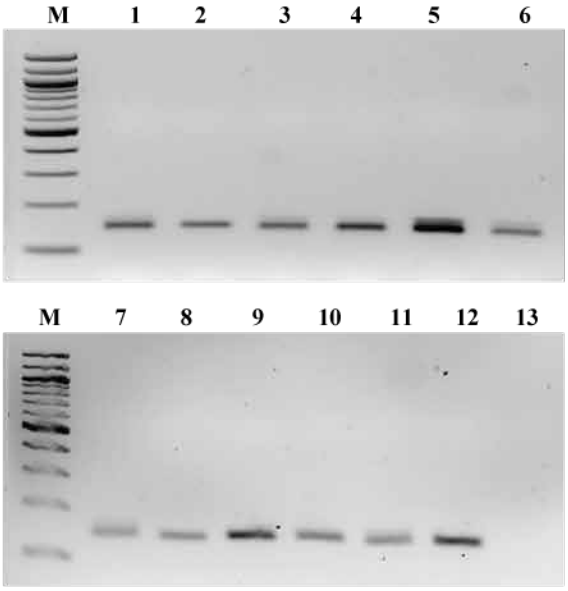

Figure 7. 2.5 % agarose gel electrophoresis (2.5%). SSR-PCR using MiIIHR20a primer set. M (marker 100 bp), lanes 1-12 (samples), lane 13 (negative control).

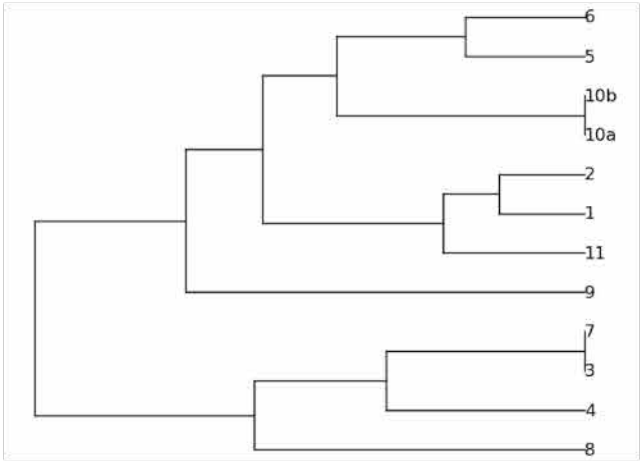

Figure 8. Dendrogram of 12 *Mangifera indica* samples constructed from RAPD pattern generated with primer N03. It was generated by the UPGMA method using PyElph 1.4 software.

Molecular markers

We were able to establish RAPD patterns from the samples using DNA extracted by the protocol described in Healey et al., 2014 and by the DNeasy Plant Mini kit. Of the four primers tested, A01 and G03 gave the best result. The other two primers must be tested again. A negative control was used for each amplification; no bands were detected in each negative control indicating no

contamination during the tests. A dendrogram was constructed using primer G03 (Figure 7). Two main clusters were observed. Further analysis is required.

The four primer sets tested for SSR analysis gave results in the expected size. Most of the samples exhibit only one band, which means they have only one allele for that marker. Sample 9 showed a slight size change when analyzed with primers mMiCIR005 and mMiCIR016 (Figures 4 and 5).

More studies are required to evaluate a higher number of samples. More molecular markers should be tested, a dendrogram generated using a larger number of samples and a complete statistical analysis including % of polymorphism and genetic diversity should be done.

REFERENCES

Azmat, M. A., Khan, A. A., Khan, I. A., Rajwana, I. A., Cheema, H. M. N., & Khan, A. S. (2016). Morphological characterization and SSR based DNA fingerprinting of elite commercial mango cultivars. *Pakistan Journal of Agricultural Sciences, 53(2),* 321–330. https://doi.org/10.21162/PAKJAS/16.2988

Azmat, M. A., Khan, I. A., Cheema, H. M. N., Rajwana, I. A., Khan, A. S., & Khan, A. A. (2012). Extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. *Journal of Zhejiang University: Science B, 13(4),* 239–243.

Healey, A., Furtado, A., Cooper, T., & Henry, R. J. (2014). Protocol: A simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods, 10(1),* 1–8. https://doi.org/10.1186/1746-4811-10-21.

Krishna, H., & Singh, S. K. (2007). Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement - A review. *Biotechnology Advances, 25(3),* 223–243. https://doi.org/10.1016/j.biotechadv.2007.01.001

National Horticulture Board. (2012, December 12). *Mango*. http://nhb.gov.in/report_files/mango/mango.htm

Rodrigues, S.M., Soares, V.L., De Oliveira, T.M., Gesteira, A.S., Otoni, W.C. & Costa, M.G. (2007). Isolation and purification of RNA from tissues rich in polyphenols, polysaccharides, and pigments of annatto (*Bixa orellana* L.). *Molecular Biotechnology, 37(3)*, 220-224.

Sharma, S. K., Rao, S. R., Joshi, G., & Anju, S. (2010). DNA Based Molecular Markers: Concept, Techniques and Comparison. *Advances in Applied Biotechnology, (September 2015)*, 1–22. Retrieved from internal-pdf://83.239.82.132/SHARMA.pdf SE - 1

Souza, I.G.B., Valente, S.E.S., Britto, F.B., de Souza, V.A.B., & Lima, P.S.C (2011). RAPD analysis of the genetic diversity of mango (*Mangifera indica*) germplasm in Brazil. *Genetics and Molecular Research 10 (4)*, 3080-3089.

Uddin, M. S., & Cheng, Q. (2015). Recent application of biotechniques for the improvement of mango research. *In Applied Plant Genomics and Biotechnology* (pp. 195-212). Woodhead Publishing.

Uddin, M. S., Sun, W., He, X., Teixeira da Silva, J. A., & Cheng, Q. (2014). An improved method to extract DNA from mango *Mangifera indica*. *Biologia (Poland), 69(2),* 133–138. https://doi.org/10.2478/s11756-013-0311-2

Viruel, M. A., Escribano, P., Barbieri, M., Ferri, M., & Hormaza, J. I. (2005). Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Molecular Breeding, 15(4),* 383–393. https://doi.org/10.1007/s11032-004-7982-x

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Epstein-Barr virus: Associated diseases

Virus de Epstein-Barr: Enfermedades asociadas

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ABSTRACT

Epstein-Barr virus (EBV) is also known as human herpes virus type 4. This virus is one of the most common viral pathogens that affects humans; at least, 90%-95% of the world's population is infected. Those who are carriers ignore that they are transmitters. EBV spreads through body fluids, particularly saliva, reason by which its illness is well known as "kissing disease". EBV is the most frequent cause of Infectious mononucleosis (IM). It usually has an asymptomatic course and it associates with periodontal, hematological and neurological diseases, primary and secondary immunodeficiencies, cancer and post-transplant infections. The objective of this review is to show the role of EBV and its association with various malignant and non-malignant diseases.

RESUMEN

Virus de Epstein-Barr (VEB) es también conocido como virus del herpes humano 4. Es uno de los patógenos virales más comunes que afectan a los humanos y al menos infecta al 90-95% de la población mundial. A menudo los portadores ignoran que son trasmisores del virus. El VEB se propaga a través de fluidos corporales, particularmente saliva, por eso se le conoce como "enfermedad del beso". El VEB es la causa más frecuente de Mononucleosis Infecciosa (MI), usualmente cursa asintomático y se asocia con enfermedades periodontales, hematológicas, neurológicas y oncológicas, inmunodeficiencias primarias y secundarias, e infecciones post-trasplantes. El objetivo de esta revisión es mostrar el papel de EBV y su asociación con diversas enfermedades malignas y no malignas.

KEYWORDS EBV, lymphoma, Infectious mononucleosis, lymphoproliferative syndrome

PALABRAS CLAVE VEB, linfoma, Mononucleosis infecciosa, síndrome linfoproliferativos

INTRODUCTION

Epstein–Barr virus (EBV) was discovered in 1964 and identified as one of the eight human herpesviruses. It is a DNA virus that could affect 90% of the human population (Sangueza & Sandoval, 2018). EBV is associated with many diseases, and was the first known tumor virus, however, it is difficult to establish its pathogenic role (AbuSalah et al., 2020; Taylor et al., 2015). EBV is usually acquired silently early in life and carried thereafter as an asymptomatic infection of the B lymphoid system. However, many circumstances disturb the delicate EBV-host balance and cause the virus to display its pathogenic potential. Thus, primary infection in adolescence can manifest as infectious mononucleosis (IM) (Dunmire et al., 2015) as immunodeficiency or as a chronic active infection with severe hemophagocytosis, when it spreads to other lymphoid lineages. Most importantly, EBV is etiologically linked to a wide range of human tumors: B cell malignancies, notably Burkitt's lymphoma, Hodgkin's lymphoma, and post-transplant lymphoproliferative disease; extranodal lymphomas of T or natural killer (T/NK) cell origin; undifferentiated nasopharyngeal carcinoma; a smooth muscle cell sarcoma and a distinct subset of gastric carcinomas (Taylor et al., 2015). IM is medically important because of the severity and duration of acute illness and also because of its long-term consequences, especially the development of certain types of cancer and autoimmune disorders (Dunmire et al., 2015).

CHARACTERISTICS OF EBV

EBV was discovered and introduced by Tony Epstein and Ivonne Barr (1964) from a Burkitt's lymphoma cell (Young & Dawson, 2014). In 1968 it was identified as the etiological agent of infectious mononucleosis, a positive heterophile infection. In1970 virus DNA was detected in tissues of positive HIV patients with nasopharyngeal carcinoma. EBV was the first human virus suggested to be oncogenic, since its DNA was found on all kind of neoplastic tissues (Tabibzadeh et al., 2020; Beltramino et al., 2005).

Later on, they described antibodies that recognized antigens from

the cell lines of Burkitt's lymphoma in African patients. Similar to Burkitt's lymphoma, antigen specific antibodies were also present in the serum of patients with post-nasal space carcinomas. Those antibodies were found in a high proportion of patients from Africa and the United States (Young & Dawson, 2014). Subsequently, Evans associated the EBV with Hodgkin's disease as a complication of infectious mononucleosis (Mueller, 1987). EBV or human gamma herpesvirus 4 (HHV-4) belongs to the 90 genus in which the only natural reservoir are humans with an incubation period of 4-8 weeks (Baumforth et al., 1999).

The virus contains linear doubled-stranded DNA that codes for more than 85 genes, surrounded by an icosahedral nucleocapsid, with 162 capsomeres, an integumentary protein between the nucleocapsid, the envelope, and an outer envelope with glycoprotein spicules (Baumforth et al., 1999).

Two subtypes of EBV are known to infect humans: EBV type-1 and EBV type-2, which differ in the organization of genes that encodes the nuclear antigens (sequence-specific DNA binding phosphoprotein, necessary for the replication and maintenance of the EBV genome, also plays a central role in the maintenance of latent EBV infection) (Beltramino et al., 2005). The virus has tropism for B cells; it enters the cells through the binding of viral envelope glycoprotein (gp) 350 to the CD21 (formerly called CR2) receptor on their surface; gp42 binds to MHC class II molecules (Beltramino et al., 2005).

The EBV genome in human cells a circular episome, has 46 functional small untranslated RNAs, and approximately 85 genes that encode proteins according to the phase of the viral life cycle (Marques-Piubelli et al., 2020).

The EBV infection process is biphasic: lytic replication and latency (EBV infection has three main latency patterns, III, II and I, Table 1 (Medina-Ortega et al., 2017)). Primary infection consists of a lytic phase that occurs because the host lacks immunity against the virus, which destroys cells at the point of entry (i.e., the oropharynx). However, over the next few days and weeks, cellular and humoral immune responses are developed, and the virus becomes latent in memory B cells. Memory B cells are the EBV reservoir in healthy individuals; latency represents a viral mechanism for eluding recognition of infected cells by the immune system (Marques-Piubelli et al., 2020).

This virus has a worldwide distribution and produces infections in immunocompromised hosts. The EBV disease has an acute and a latent phase; in the first one its classical manifestation is the infectious mononucleosis and in the second one the patient becomes a reservoir for life. The virus is associated with both, non-malignant diseases and a number of human cancers. Also, it is one of the most common herpes to get infected from, since at least 90-95% of the world population has been infected by the virus (Jarrett, 2010; Pei et al., 2017).

EBV infection commonly occurs during childhood and is asymptomatic. Furthermore, EBV infection in adolescents and young adults leads to IM in 35% to 50% of cases (Pei et al., 2017). The incidence is highest between 15 and 24 years of age; over 90% of adults worldwide are seropositive for EBV antibodies at 35 years of age and the incidence is even higher in first year university students (Womack & Jimenez, 2015).

Table 1. Types of latency, EBV molecules and associated malignancies.

Latency Type	Molecules expressed	Associated types of cancer
I	EBNA-1, EBER	Gastric carcinoma Burkitt lymphoma Diffuse large B-cell lymphoma
II	EBNA- 1, EBER, LMP-1,2,3	Hodgkin lymphoma T / NK cell lymphomas Nasopharyngeal carcinoma
III	EBNA, EBER, LMP	Post-transplant LPD Lymphomas associated with HIV / AIDS

EBER: EBV- encoded small RNAs, EBNA: EBV nuclear antigens, LMP: Latent membrane proteins, Post-transplant LPD: Post-transplant lymphoproliferative diseases

EBV is present in body fluids, mainly saliva, and is therefore transmitted through coughing, food exchange, and kisses (hence the layman's term "deceased kisses"), eating utensils, toothbrushes and having contact with toys that children have drooled on (Fugl & Andersen, 2019; CDC and Prevention, 2018).EBV targets B cells and a wide range of cells particularly in those of epithelial lineage. Pharyngeal infection is followed by dissemination of virus throughout the body, with B cells as its primary target. The immune response mounts steadily, with expansion of EBV-specific cytotoxic T-cell clones, eventually recognizing and controlling the primary infection. Control of EBV proliferation is signaled by a shift from lytic viral activity to a latent phenotype in an immortalized B lymphocyte pool, which provides a lifelong source of low-grade reactivation. The serological response provides reliable markers for acute and chronic infection in immunocompetent hosts, with initial IgM and IgG to viral capsid antigen, followed by antibody to the EBV nuclear antigen developing months after infection (Nowalk & Green, 2016).

EBV can cause severe and acute malignant tumors of lymphoid or epithelial origin under immunosuppressed conditions (Andrei et al., 2019). EBV is associated with the development of B-cell malignancies, such as Hodgkin's lymphoma, nasopharyngeal carcinoma, Burkitt's lymphoma and other non-Hodgkin lymphoma lung carcinoma, X-linked lymphoproliferative syndrome, diffuse large B-cell lymphoma, HIV B-cell lymphoma,

hemophagocytic lymphohistiocytosis, NK/T cell lymphomas, post-transplant lymphoproliferative disorder (Pei et al, 2017).

EBV initially infects epithelial cells in the oropharynx (its gateway into the body) and then passes to B cells in adjacent lymphoid tissue. The primary infection that this virus induces marks the immune response against viral capsid antigens (VCA) that have the ability to neutralize and prevent generalized viremia. Lytic infection is associated with the expression of a large number of viral genes, the production of virions and the infected cell's death; in contrast, latent infection is associated with the expression of a small number of EBV genes, persistence of the infection, and growth transformation (Jarrett, 2010).

Expression of the complete set of latent genes is known as type III latency and is associated with transformation of B cells. Expression of the EBV gene in EBV positive lymphomas that occur in the context of immunosuppression often follows this pattern; however, more restricted patterns of EBV gene expression are also observed. In EBV associated Hodgkin' lymphoma, Hodgkin and Reed/Sternberg (HRS) cells are infected with EBV and the infection is clonal, that is, all tumor cells are derived from a single infected cell (Jarrett, 2010).

EBV AND INFECTIOUS MONONUCLEOSIS (IM)

IM is a viral disease that classically presents the triad of fever, lymphadenopathy, and pharyngitis. EBV causes 90% of acute cases. Most adults are positive for EBV antibodies, and most patients gain exposure in early childhood without ever showing any symptoms, but single exposure to EBV confers lifelong immunity. The typical acute presentation involves a patient in mid to late adolescence; acute infection is rare in patients over 30 years. After being infected, patients will shed the virus for months. Incubation period typically is between 1 and 2 months (Womack & Jimenez, 2015; Lennon et al, 2015).

Acute symptomatic phase lasts 2–4 weeks, fever, typically is low grade. Chills, sweats, myalgia, and arthralgia are rare; lymphadenopathy may be diffusing, but classically the patient has palpable posterior cervical lymph nodes. Patients usually have notable pharyngitis and tonsillar swelling. Tonsillar exudates and palatal petechial can mimic the appearance of an acute streptococcal infection. Fatigue is one of the most common symptoms, and, in spite it generally is limited to the acute phase, it may persist for weeks or even months after other symptoms resolve. Splenomegaly is also common and, in a few patients, may be the presenting symptom. Nausea, vomit, rash, and headache are frequent, with other symptoms such as oral hairy leukoplakia, facial edema, jaundice, and hepatomegaly occurring in a minority of patients (Lennon et al., 2015; Schwartzkopf, 2018).

A thorough medical history and physical examination are important for accurate clinical diagnosis. Laboratory testing includes the rapid monospot test, which detects the presence of heterophile antibodies and is highly specific for EBV. Sensitivity is decreased early in the illness but improves after the first week of acute infection and peaks at 2–5 weeks. EBV-specific antibody testing is available, but generally should be reserved for patients with chronic or persistent symptoms in which the diagnosis of mononucleosis might be uncertain. Lymphocyte count may be elevated with atypical lymphocytes greater than 10%; there is also total leukocytosis with elevated white blood cells up to 20,000 cells/mm^3. Thrombocytopenia is rare (Lennon, et al., 2015; Schwartzkopf, 2018).

EBV INFECTION AND NEOPLASM'S DEVELOPMENT

Epstein– Barr virus in epithelial malignancies

EBV has a complex role in the development of non-keratinizing nasopharyngeal carcinomas. This is explained by the oncogenic latent genes of the virus, which cause primary transformation of epithelial cells. The proliferation action of EBV is not the central mechanism leading to associated cancers. Most of the genes altered by EBV infection have immune evasion activities; associated malignancies have anti-apoptotic properties. Host cells modulate the gene expression that alters the growth properties and induces carcinogenesis (Tsang & Tsao, 2015).

EBV and Nasopharyngeal carcinoma, Non-Hodgkin's lymphoma and Gastric carcinoma

Nasopharyngeal carcinoma is an epithelial cancer. Several factors are important in the pathogenesis, one of these factors, is indeed EBV. The mechanism in this case is EBV altering the cell's genes, and later on, immortalizing the cell (Young & Dawson, 2014; Roberts et al., 2015).

The undifferentiated histological type of nasopharyngeal carcinoma is the narrow association between human tumors and EBV infection. EBV is found in Hodgkin's disease in approximately 35% of the patients, in gastric carcinomas approximately 10% and in all cases of non-keratinizing nasopharyngeal carcinoma. The type 2 and the type 3 histological types of nasopharyngeal carcinoma, according to the World Health Organization (WHO), are predominantly EVB-positive (Tsao et al., 2017).

Novel therapeutic approaches use virus reactivation, gene therapy, or therapeutic vaccination for our ability to effectively target EBV-associated carcinomas (Young & Dawson, 2014). The recurrent localization of EBV positive mucocutaneous ulcer (EBVMCU) in the oral cavity and gastrointestinal tract probably reflects the initial site of EBV inoculation in the oropharynx and the persistence of latent EBV within the lymphocytes of the Waldeyer ring and the lymphoid tissue associated with the intestine (Chen et al., 2015).

Researchers suggest that EBVMCU is a debilitating and persistent disorder. For the treatment of this pathology, aggressive therapy options are required. Antibody therapy against CD20 and CD30, local radiotherapy, surgery, chemotherapy, and a combination of these, have been used to treat EBVMCU with high rates of persistent clinical remission (Chen et al., 2015).

EBV AND LYMPHOPROLIFERATIVE DISORDERS

EBV can infect B cells and establish a latent infection, further inducing lymphomagenesis under specific microenvironment conditions (Pei et al., 2017). In reality, the pathogenesis of EBV associated lymphomas involves a complex interaction between different virus patterns, gene expression, and cellular genetic changes (Shannon et al., 2017). There are several well-described forms of EBV latency, each of which the virus uses at different stages of the viral life cycle and which are also shown in the latency patterns observed in various neoplasms associated with EBV (Young & Dawson, 2014; Medina et al., 2017).

In the past 30 years, several primary immunodeficiencies related to a high risk to develop EBV-associated lymphoproliferative disorders (LPDs) have been characterized. Among those are included, virus-associated hemophagocytic syndrome, non-malignant and malignant B-cell LPDs including non-Hodgkin and Hodgkin's types of B lymphomas (Latour & Winter, 2018).

As part of the associated diseases, there are also late complications in the presence of EBV and IM. Lymphoproliferative cancers, which are the most established late-onset complication for IM, have been investigated in several different cohorts since the 1970s. A 2003 Scandinavian study found an increased risk of EBV-positive Hodgkin's lymphoma in young adults, and a mean incubation period of 4.1 years with a maximum risk after 2.1 years after primary infection (Fugl & Andersen, 2019).

The carcinogenic properties of EBV were first raised in 1964, when EBV was detected in cultured tumor cells from Burkitt's lymphoma patients in tropical Africa. Since then, this highly aggressive type of B-cell lymphoma has been strongly correlated with EBV. The EBV status is unaffected by the treatment regimen, which consists of high-intensity chemotherapy and anti-CD20 monoclonal antibody therapy in fit patients resulting in remission in >85% of cases (Fugl & Andersen, 2019). EBV has also been associated with diffuse large B-cell lymphoma (DLBCL) in 10% of cases among immunocompetent patients. EBV-positive DLBCL appears to primarily affect elderly patients and is now called EBV (+) DLBCL, not otherwise specified (NOS). This state has confirmed to affect the prognosis since these patients had a poor responder to conventional therapy (Fugl & Andersen, 2019).

Burkitt's lymphoma

Burkitt's lymphoma is a highly aggressive B cell non-Hodgkin lymphoma characterized by the translocation and deregulation of the MYC gene on chromosome 8 with the potential to involve multiple organ systems. Three subtypes of Burkitt's lymphoma sporadic, endemic, and immunodeficiency-associated exist, and they all present different epidemiology, risk factors, and clinical presentations. Since the discovery in 1964 of EBV in African Burkitt's lymphoma, it was associated with a remarkably diverse range of cancer types. B, T, and NK cell-originating lymphoproliferative lesions and malignant lymphomas (Shannon et al., 2017; Vockerodt et al., 2014). As a result, there are three main types of EBV-related B-cell malignancies: Burkitt's lymphoma, Hodgkin's lymphoma, and diffuse large B-cell lymphomas (Shannon et al., 2017).

Burkitt's lymphoma occurs not only in its endemic form, but also at a lower incidence worldwide known as "sporadic" Burkitt's lymphoma, and in an HIV-associated form. In acquired immunodeficiency syndrome (AIDS), B-cell lymphomas have been described with evidence of latent EBV infection and chromosomal translocation typical of Burkitt's lymphoma, as well as other types of non-Hodgkin lymphomas (Ho et al., 1988). All forms of Burkitt's lymphoma harbor a reciprocal chromosomal translocation that affects the MYC gene on chromosome 8 and one of the immunoglobulin heavy chain loci or κ and λ of the light chain on chromosome 14, 2 or 22, respectively (Vockerodt et al., 2014). Antibody titers against the major determinants of EBV have included the viral capsid antigen (VCA). Antibodies against VCA are detected as form of EBV infection's evidence. Furthermore, antibodies against early antigen are present during primary infection and reactivation. Two components of early antigen antibodies can be distinguished: the diffuse (D) elevated in nasopharyngeal carcinoma and the restricted (R) elevated in Burkitt's lymphoma (Mueller, 1987). A final determinant is nuclear antigen (EBNA), which is a virus-induced antigen expressed in lymphocytes that carry the EBV genome, "transformed" B cells (Mueller, 1987).

Burkitt's lymphoma can be classified into three forms according to geographical distribution: endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma and Burkitt's lymphoma associated with HIV (Pei et al., 2017). The discovery of EBV in Burkitt's lymphoma tumors and the fact that almost 100% of endemic Burkitt's lymphomas are positive for EBV support the possibility that Burkitt's lymphoma tumors are driven by EBV as a major contributor (Pei et al., 2017). Furthermore, epidemiological studies in serum have provided evidence that African Burkitt's lymphoma tumors are positive for EBV. A critical feature of Burkitt's lymphoma tumors are MYC translocation and activation. Overexpression of MYC in Burkitt's lymphoma deaths resulting from a translocation between the MYC gene and the immunoglobulin locus that further regulates tumorigenesis. Most EBV positive Burkitt's lymphoma tumors consistently express the EBNA1 latent antigen as the predominant latent antigen and are called latency I (Table 1) (Medina et al., 2017).

Hodgkin's lymphoma

Hodgkin's disease is an unusual malignancy characterized by the presence of a minority of malignant Hodgkin/Reed Sternberg cells surrounded by a non-neoplastic inflammatory infiltrate. Recent studies suggest that one of the two distinct forms of Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL), harbors a timy proportion of EBV. EBV is believed to have a casual paper in some cases of Hodgkin's lymphoma. The parallelism of the peak of the incidence by age curve in Hodgkin's lymphoma's and IM reinforced the relationship between EBV and Hodgkin's lymphoma (Alexander et al., 2000).

The seminal papers published in 1957 and 1966 suggested that Hodgkin's lymphoma in younger and older adults had different etiologies and suggested an infectious etiology for Hodgkin's lymphoma in young adults. EBV infection can be lytic or latent. Lytic infection is associated with the expression of a large number of viral genes, the production of the progeny virus and the death of the infected cell. In contrast, latent infection is associated with the expression of a small number of EBV genes, persistent infection, and growth transformation (Jarrett, 2002).

In the absence of prevention of EBV infection, it is difficult to demonstrate that the association between EBV and classic Hodgkin's lymphoma is casual. Although, there are three separate lines of evidence linking EBV to Hodgkin's lymphoma: (1) a history of IM in Hodgkin's lymphoma cases (2); serological studies comparing antibody titers against EBV in cases and (3) Hodgkin's lymphoma controls (Massini et al., 2009).

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is an aggressive neoplasm. This lymphoma is the most common type of lymphoma. It accounts for one third of all lymphoma cases in one of the variants. The 10% of the cases are EBV-positive diffuse large B-cell lymphoma (EBV-positive DLBCL), which can present different latency patterns and are related to the host immune system status (Marques-Piubelli et al., 2020; Pei et al., 2017; Vockerodt et al., 2014; Sukswai et al., 2020).

The disease was denominated at first as "senile EBV associated B-cell lymphoproliferative disorder", but various studies have shown that EBV-positive DLBCL also can arise in younger people even children (Marques-Piubelli et al., 2020; Pei, Lewis et al., 2017; Vockerodt et al., 2014; Sukswai et al., 2020).

In some cases, medium to large neoplastic with Hodgkin/Reed-Sternberg-like and lymphocyte-predominant–like cells are scattered in a reactive infiltrate of small lymphocytes, histiocytic, and plasma cells. This appearance, designated by some as the polymorphous subtype, is the most frequent. There are lesions of the blood vessels with coagulative necrosis (Marques-Piubelli et al., 2020).

EBV is commonly present in DLBCL when it coexists with an immunodeficiency. EBV-positive DLBCL represent 30-50% of late-onset post-transplant tumors, and about 30% of AIDS-DLBCL cases, many of which are the inmunoblasic type. Recognition that EBV-positive DLBCL may as well be present in elderly patients with no medical history of immunesuppression has caused the disease to acquire the name "EBV-positive diffuse large B-cell lymphoma" (Vockerodt et al., 2014). DLBCL prevails as one of the scarce lymphomas that remains curable due to the scientific advances made. Above 50% can be cured by chemo-, radio-, or immunotherapy regimens. The challenge is treating those who develop a recurrent or refractory (Pei et al., 2017). EBV-positive DLBCL activates NF-κB and JAK/STAT signaling pathways, but detailed mechanisms of lymphomagenesis persists unknown (Shannon et al., 2017).

Epstein-Barr virus-positive mucocutaneous ulcer (EBVMCU)

It is recently recognized that B-cell lymphoproliferative disorder that is driven by a latent EBV infection, causes discrete ulcerations in the gold pharynx, gastrointestinal tract, and skin. The diagnosis is based on clinical, morphological, and immunophenotypic features. The histological pattern is usually described as a cutaneous or mucosal ulcer with occasional pseudoepitheliomatous hyperplasia in the adjacent epithelium (Marques-Piubelli et al., 2020; Roberts at al., 2015).

NK/T cell lymphomas

Other diseases associated with EBV are NK/T cells extranodal lymphoma (ENKTL) non-B cell lymphomas (Vockerodt et al., 2014). ENKTL is a rare and aggressive tumor frequent in Asian and Latin American populations (Medina et al., 2017). ENKTL cells produce cytokines responsible for the disease's symptoms of these tumors and for disorders related to EBV (CAEBV) and fatal hemophagocytic syndrome. ENKTL express a latency pattern II with variable expression of LMP1 (Medina et al., 2017), which contributes to the excessive production of pro inflammatory cytokines mediated by the activation of NF-κB (Vockerodt et al., 2014).

EBV is B cell tropic, and is unable to infect the T and NK cells. However, ectopic T cell or NK infection occurs rarely, which leads to EBV-driven lymphoproliferations clinically manifested as infection or monoclonal neoplasms. ENKTL, aggressive NK leukemia (ANKL) and 25% of CAEBV cases progress to lymphoma. ENKTL is an uncommon but aggressive kind of EBV-associated non-Hodgkin lymphoma (Shannon et al., 2017).

ENKTL is characterized by extensive necrosis and angio invasion and generally occurs in extra nodal sites within the upper aero

digestive tract, nasal cavity, nasopharynx, paranasal sinus and palate. Other sites are the skin, respiratory tract, gastrointestinal tract, and testicles. The disease occurs mainly in 40-50-year-old adults, mainly in men. ENKTL accounts for up to 1% of non-Hodgkin's lymphoma cases in North America or Europe (Shannon et al., 2017).

EBV INFECTION PREDISPOSED TO HEMATOLOGICAL DISEASES

Hemophagocytic lymphohistiocytosis

EBV can also cause hemophagocytic disease, or EBV-associated hemophagocytic lymphohistiocytosis (HLH) (Dunmire et al., 2015). There is a relative rarity barrier to the diagnosis of HLH and EBV-HLH cases are even more rare. The disease is characterized by fever, splenomegaly, cytopenias high levels of ferritin and soluble CD25 (Dunmire et al., 2015).

X-linked lymphoproliferative syndrome

The X-linked lymphoproliferative syndrome (XLP) is a disease characterized by anemia, hypergammaglobulinemia, and lymphohistiocytosis. In general, children with this disease exhibit massive cellular responses to primary EBV infection. That causes hemophagocytic pathology; a burden that EBV-transformed B cells cannot automatically control (Dunmire et al., 2015; Balfour et al., 2015).

The researchers described an X-linked lymphoproliferative disease (XLP) in children who typically present fulminant infectious mononucleosis with lymphocytic and histiocytic infiltration of the bone marrow, central nervous system, and other organs. Subsequent studies mention that the disease has a variety of phenotypes including fatal infectious mononucleosis, aplastic anemia or hypogammaglobulinemia after primary EBV infection, immunoblastic B-cell lymphoma, Burkitt lymphoma, or plasmacytoma. The underlying cause is an abnormal immune response to EBV infection (Cohen, 2015).

The main deficiency involved with XLP was found to be in the protein associated with the signaling lymphocytic activation molecule (SAP), which is encoded by the human gene *SH2D1A* (Dunmire et al., 2015). Chronic Epstein-Barr Virus (CAEBV) infection is rare and mainly limited to Japan and East Asia, but has gained international vigilance due to the increase in cases worldwide. Additional CAEBV is rare, a clinician should be aware of this as a differential diagnosis in patients with persistent MI symptoms for more than 3 months (Fugl & Andersen, 2019). The first genetic disorder related to severe and often fatal EBV disease is XLP1, also known as Duncan's disease (Cohen, 2015).

In updated studies, there is evidence that the presenting symptoms of XLP1 include haemophagocytic lymphohistiocytosis (HLH) in 31.9% of people, dysgammaglobulinema in 22%, family history of XLP1 only in 16.5%, lymphoma in 14.3%, fulminant infectious mononucleosis in 7.7 %, and other symptoms at 7.7% (Cohen, 2015).

X-linked lymphoproliferative syndrome also has an increased risk of complications from EBV infection, and two thirds die from the infection. As these patients are immunocompromised, those with X-linked lymphoproliferative disorders have the highest risk of fulminant EBV infection (Womack & Jimenez, 2015).

EBV IN IMMUNOCOMPROMISED PATIENTS

The incidence of EBV-associated lymphomas increases if the patients have the Human Immunodeficiency Virus (HIV). The incidence of the CD4+ immunoblastic DLBCL increases (80%) only when the amount of circulating T cells decreases, therefore the patient is severely immunocompromised (Pei et al., 2017; Vockerodt et al., 2014).

The increased reports of EBV-associated lymphomas with the onset of acquired immunodeficiency syndrome (AIDS) implies a molecular connection of EBV in HIV infected hosts. Almost all the lymphomas that are associated to AIDS and EBV express latent antigens and a type III latency schedule. This is likely due to a weak immune system and control loss of EBV infected cells (Pei et al., 2017; Medina et al., 2017).

FREQUENCY OF EBV INFECTION BY POST TRANSPLANTATION

EBV infections are commonly observed in patients after orthotropic liver transplantation and serologic evidence of active disease is demonstrable in 24% of cases. Frequently is an asymptomatic infection. However, 1-2% of transplant recipients develop persistent or recurrent disease culminating in the development of post-transplant lymphoproliferative disorders, some of which behave like aggressive lymphomas (Randhawa et al., 1990).

Post-transplant lymphoproliferative disorder

Post-transplant lymphoproliferative disorder (PTLD) has its origins from B cells in transplant patients. It is mostly associated with EBV infection in the chaining of an impaired immune care system. At the same time, 60-80% of PTLDs show to be EBV positive (Parvaneh et al., 2013). EBV is the decisive promoter of the development of PTLD that generally occurs in cases of early transplant initiation. Early-onset PTLDs that are associated with EBV-infected B cells are usually polyclonal or oligo clonal, while most late-onset PTLDs with or without EBV infection are monoclonal (Parvaneh et al., 2013). Transplant-associated immunosuppression in PTLDs leads to the expression of EBNA3 family members in addition to all latent antigens, which are

characteristic of EBV infection associated with latency III (Table 1) (Medina et al., 2017). Prevention and treatment of EBV-associated PTLDs depend on surgery with irradiation, immunotherapy with monoclonal antibodies (for example, Rituximab), and antiviral medications (Parvaneh et al., 2013).

About 92% of transplant recipients were seropositive for EBV before transplantation and 8% were seronegative. After transplantation, the EBV reactivation rate among seropositive recipients was 33%, and the primary infection rate among seronegative recipients was 82% (Ho et al., 1988). 8% of EBV-seronegative children and 10.5% of children with primary infection develop lymphoproliferative syndromes (Ho et al., 1988).

In healthy asymptomatic carriers, EBV infection is suppressed by an EBV-specific immune response. An incompetent immune response leads to unmeasured expansion of EBV-transformed B cells (i.e., tumors that appear shortly after hematopoietic or solid organ stem cell transplantation in highly immunosuppressed patients) (Vockerodt et al., 2014).

Lymphoproliferative syndrome

EBV-associated lymphoproliferative syndromes are highly aggressive and lethal in transplant patients (Ho et al., 1988). EBV infections are silent even in immunodepressed individuals, but a small but significant proportion of patients develop lymphoproliferative syndromes (Balfour et al., 2015).

DISCUSSION

In conclusion, this review seeks to highlight recent advances in understanding EBV, focusing on exposing the causes to acute illnesses, such as the long-term consequences as a result of virus acquisition. The key challenges in the future are the development of protective vaccines and new effective treatments since EBV is one of the most important human pathogens throughout history. Although this virus was discovered more than 50 years ago and infected more than 90% of the population worldwide, even so, there are large gaps in our knowledge of its epidemiology and pathogenesis (Dunmire et al., 2015; Balfour, et al., 2015).

The role of EBV in cancer development has been well documented worldwide. EBV is associated with the development of B-cell malignancies, such as: Hodgkin's lymphoma, nasopharyngeal carcinoma (NPC), Burkitt's lymphoma, other non-Hodgkin's lymphomas, lymphoproliferative cancer (LPC), diffuse large B-cell lymphoma (DLBCL), HIV B-cell lymphoma, hemophagocytic lymphohistiocytosis (HLH), NK/T cell lymphomas, X-linked lymphoproliferative syndrome (XLP), post-transplant lymphoproliferative disorder (PTLD) and lymphoproliferative syndrome (LPS), among many others. On the other hand, it is necessary to increase efforts in the investigation of this problem in our country and worldwide, due to the great social, economic and health impact that it will have (Dunmire et al., 2015; Balfour et al., 2015).

As it was clear throughout this article, there are more doubts than answers. Future challenges are to focus research on how EBV is transmitted, the development of a treatment and the creation of an EBV vaccine. In terms of EBV-associated cancer, we know a reasonable amount about how this virus infects and transforms lymphocytes and epithelial cells (Dunmire et al., 2015; Balfour et al., 2015).

Antivirals (acyclovir, valomaciclovir, and valacyclovir) have been proposed for some time as therapeutic methods to treat EBV infection, nonetheless no evidence supports these treatments. In the meanwhile, therapeutic should concentrate on treating the symptoms. In severe cases, the patient requires hospitalization for intravenous fluids due to dehydration, or monitoring to avoid respiratory failure (Fugl & Andersen, 2019; Lennon et al., 2015).

The medical community suggests an intimate relationship between EBV and various lymphomas, especially Hodgkin's lymphoma, and it is for this reason that this association is believed to be casual. Proteins encoded by EBV are expressed and have a plausible role in the pathogenic process (Fugl & Andersen, 2019; Lennon et al., 2015).

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REFERENCES

AbuSalah, M.A.H., Gan, S.H., Al-Hatamleh, M.A.I., Irekeola, A.A., Shueb, R.H., & Yean Yean, C. (2020). Recent Advances in Diagnostic Approaches for Epstein-Barr Virus. *Pathogens, 18;9(3)*. pii: E226.

Alexander, F.E., Jarrett, R.F., Lawrence, D., Armstrong, A.A, Freeland, J., Gokhale, D.A., Kane, E., Taylor, G.M., Wright, D.H., & Cartwright, R.A. (2000). Risk factors for Hodgkin's disease by Epstein-Barr virus (EBV) status: prior infection by EBV and other agents. *British Journal of Cancer, 82(5),* 1117-1121.

Andrei, G., Trompet, E., & Snoeck, R. (2019). Novel Therapeutics

for Epstein–Barr Virus. *Molecules, 24(5),* 997.

Balfour, H.H., Dunmire, S.K., & Hogquist, K.A. (2015). Infectious mononucleosis. *Clinical & Translational Immunology, 4(2).*

Baumforth, K.R., Young, L.S., Flavell, K.J., Constandinou, C., & Murray, P.G. (1999). The Epstein-Barr virus and its association with human cancers. *Molecular Pathology, 52(6),* 307–322.

Beltramino, M. P., Calmet, R., & Gatica Valdes, M. (2005). Virus de Epstein-Barr y su relación con el desarrollo de enfermedades linfoproliferativas. *Hematología, 9(2),* 39–54.

Chen, X., Kamranvar, S. A., & Masucci, M. G. (2015). Oxidative stress enables Epstein–Barr virus-induced B-cell transformation by posttranscriptional regulation of viral and cellular growth-promoting factors. *Oncogene, 35(29),* 3807–3816.

Centers for Disease Control and Prevention. (2018). Epstein-Barr Virus and Infectious Mononucleosis. USA: Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/epstein-barr/index.html.

Cohen, J.I. (2015). Primary Immunodeficiencies Associated with EBV Disease. *Current Topics in Microbiology and Immunology Epstein Barr Virus Volume 1,* 241-265.

Dunmire, S.K., Hogquist, K.A., & Balfour, H.H. (2015). Infectious Mononucleosis. *Current Topics in Microbiology and Immunology Epstein Barr Virus Volume 1*, 211-240.

Fugl, A., & Andersen, C. L. (2019). Epstein-Barr virus and its association with disease - a review of relevance to general practice. *BMC Family Practice, 20(62).*

Ho, M., Jaffe, R., Miller, G., Breinig, M.K., Dummer, J.S., Makowka, L., Atchison, R.W., Karrer, F., Nalesnik, M.A., & StarzI, T.E. (1988). The Frequency of Epstein-Barr Virus Infection and Associated Lymphoproliferative Syndrome After Transplantation and Its Manifestations in Children. *Transplantation, 45(4),* 719-726.

Jarrett, R.F. (2002). Viruses and Hodgkins lymphoma. *Annals of Oncology, 13*, 23-29.

Jarrett, R.F. (2010). The Role of Viruses in the Genesis of Hodgkin Lymphoma. *Hodgkin Lymphoma,* 21-32.

Latour, S., & Winter, S. (2018). Inherited Immunodeficiencies With High Predisposition to Epstein–Barr Virus-Driven Lymphoproliferative Diseases. *Frontiers in Immunology, 9,* 1103.

Lennon, P., Crotty, M., & Fenton, J.E. (2015). Infectious mononucleosis. *British Medical Journal, 350,* h1825.

Marques-Piubelli, M. L., Salas, Y. I., Pachas, C., Becker-Hecker, R., Vega, F., & Miranda, R. N. (2020). Epstein–Barr virus-associated B-cell lymphoproliferative disorders and lymphomas: a review. *Pathology, 52(1),* 40-52.

Massini, G., Siemer, D., & Hohaus, S. (2009). EBV in Hodgkin lymphoma. *Mediterranean Journal of Hematology and Infectious Diseases, 1(2),* e2009013.

Medina-Ortega, A.P., López-Valencia, D., Mosquera-Monje, S.L., Mora-Obando, D.L., & Dueñas-Cuéllar, R.A. (2017). Virus de Epstein-Barr y su relación con el desarrollo del cáncer. *Iatreia, 30(2),*131-145.

Mueller, N. (1987). Epidemiologic Studies Assessing the Role of the Epstein-Barr Virus in Hodgkin's Disease. *The Yale Journal of Biology and Medicine, 69,* 321–327.

Nowalk, A., & Green, M. (2016). Epstein-Barr Virus. *Microbiology Spectrum, 4(3).*

Parvaneh, N., Filipovich, A.H., & Borkhardt, A. (2013). Primary immunodeficiencies predisposed to Epstein-Barr virus-driven haematological diseases. *British Journal of Haematology, 162(5),* 573-586.

Pei, Y., Lewis, A.E., & Robertson, E.S. (2017). Current Progress in EBV-Associated B-Cell Lymphomas. *Advances in Experimental Medicine and Biology, 1018,* 57-74.

Randhawa, P. S., Markin, R.S., Starzl, T.E., & Demetris, A.J. (1990). Epstein-Barr Virus—Associated Syndromes in Immunosuppressed Liver Transplant Recipients. *The American Journal of Surgical Pathology, 14(6),* 538-547.

Roberts, T.K., Chen, X., & Liao, J.J. (2015). Diagnostic and therapeutic challenges of EBV-positive mucocutaneous ulcer: a case report and systematic review of the literature. *Experimental Hematology & Oncology, 5(1).*

Sangueza-Acosta M., & Sandoval-Romero E. Epstein-Barr virus and skin. (2018). *Brazilian Annals of Dermatology, 93(6),* 786-799.

Schwartzkopf, J. (2018). Infectious mononucleosis. *Journal of the American Academy of Physician Assistants, 31(11),* 52-53.

Shannon-Lowe, C., Rickinson, A.B., & Bell, A.I. (2017). Epstein–Barr virus-associated lymphomas. *Philosophical Transactions of*

the Royal Society B: Biological Sciences, 372(1732), 20160271.

Sukswai, N., Lyapichev, K., Khoury, J.D., & Medeiros, L.J. (2020). Diffuse large B-cell lymphoma variants: an update. *Pathology, 52(1),* 53-67.

Tabibzadeh, A., Karbalaie Niya, M.H., Esghaei M., Bokharaei-Salim F., Ataei-Pirkooh, A., Kiani S.J., & Monavari S.H.R. (2020). Molecular Epidemiology of Epstein-Barr virus (EBV) in Patients with Hematologic Malignancies. *Asian Pacific Journal of Cancer Prevention, 21(3),* 693-698.

Taylor, G.S., Long, H.M., Brooks, J.M., Rickinson, A.B., & Hislop, A.D. (2015). The immunology of Epstein-Barr virus-induced disease. *Annual Review of Immunology, 33,* 787-821.

Tsang, Chi., & Tsao, Sai. (2015). The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. *Virologica Sinica, 30(2),* 107-121.

Tsao, S.W., Tsang, C.M., & Lo, K.W. (2017). Epstein–Barr virus infection and nasopharyngeal carcinoma. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 372(1732),* 20160270.

Vockerodt, M., Yap, L-F., Shannon-Lowe, C., Curley, H., Wei, W., Vrzalikova, K., & Murray, P.G. (2014). The Epstein-Barr virus and the pathogenesis of lymphoma. *The Journal of Pathology, 235(2),* 312-322.

Womack, J., & Jimenez, M. (2015). Common questions about infectious mononucleosis. *American Family Physician, 91(6),* 372-6.

Young, L. S., & Dawson, C. W. (2014). Epstein-Barr virus and nasopharyngeal carcinoma. *Chinese Journal of Cancer, 33(12),* 581.

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OTRAS INVESTIGACIONES EN EL CAMPUS/ OTHER RESEARCH ON CAMPUS

Resumen de otras investigaciones realizadas por nuestros estudiantes en el Recinto durante el año académico 2019-2020 en los campos de farmacología-bioquímica y ecología.

Summary of other research conducted on campus by our students during the academic year 2019-2020 in the fields of pharmacology-biochemistry and ecology.

FARMACOLOGÍA-BIOQUÍMICA/ PHARMACOLOGY-BIOCHEMISTRY

1-A Evaluation of the pharmacological action of synthetic and natural derived chemical compounds on the proliferation of AsPc-1 and PANC-1 cells

Isabeliz Seijo, Fabián Mercado, Neisha Ramírez, Melanie Cubano, José Raúl Rivera, and Karen Woolcock

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent neoplastic disease of the pancreas. PDAC is resistant to radiotherapy, molecularly targeted therapy, and to the most common treatment when the disease is at advance stages, gemcitabine. Evaluating new pharmacological approaches, including the use of natural compounds is needed to achieve pancreatic cancer remission, improving survival rates and patient prognosis. In this study it was evaluated the effect on of EHop-16, a Rac inhibitor, on AsPC-1 cell proliferation. Also, 6-Shogaol, a natural compound, and derivates from benzimidazoles, known to have anti-cancer activity, were evaluated. It was found that Gemcitabine, the current treatment for pancreatic cancer, reduced AsPC-1 viability only to 63% and that the compound with highest efficacy for reducing cell proliferation was EHop-16. According to the determined GI50, the potency of EHop-16 (15.6 M) for reducing AsPC-1 was slightly higher than 6-Shogaol (18.9 M). When the effect of the tested compounds was compared with previous results obtained for PANC-1 cells, it was observed that 6-Shogaol was more potent for inhibiting the proliferation of AsPC-1 cells. Among the tested benzimidazoles, compound A showed the highest efficacy for reducing PANC-cell viability. The effect was dose-dependent and time dependent, reaching the lowest cell viability (55.7%) after exposing cells for 96 hours. The results obtained in two invitro models (PANC-1 and AsPC-1) shows that the use of the family of Rac inhibitors, such as EHop-16, may have a clinical potential, since the presented data demonstrates that this compound has higher efficacy thanthe current treatment, gemcitabine.

1-B Migration pattern analysis of adenocarcinoma pancreatic PANC-1 cells after treatment with EHop-16 and 6-Shogaol

Ivanies González, Carla Mercado, Mariela Rosario, Myraida Toledo, Kevin Ongay, and Karen Woolcock

Pancreatic cancer is a lethal malignancy and the fourth leading cause of cancer related death. Early diagnosis is difficult because most patients have no symptoms and there are no specific screening tests that can detect pancreatic cancer. Metastasis is a major cause of morbidity and mortality in patients with pancreatic cancer, and requires angiogenesis, epithelialmesenchymal transition, migration, invasion to surround tissues, formation of a pre-metastatic niche, and growth at the metastatic site. The objective of this study was to analyze the migration pattern of PANC-1 cells in presence of synthetic and natural chemical compounds, EHop-16 and 6-Shogaol. For this a Wound Healing Migration Assay was performed using a monolayer of PANC-1 cells grown in 6-well plate, with a system of coordinates. Results shows that treatment with 6-Shogaol (10 µM) slightly increased the wound recovery up to 30% after 4 hours. This represents an increase in recovery of 22.4% and suggest that 6-Shogaol may increase migration of pancreatic cells or facilitate cytoskeletal rearrangements. When cells were treated with EHop-16 (10 mM),

the wound only recovered by 8%. This represents a reduction of the recovery of 67.3% as compared with the control, strongly indicating that the migration of the pancreatic cancer cells was reduced. Since previous results obtained in the lab, has shown that EHop-16 also reduces cell proliferation of PANC-1 and AsPC-1 cells, increases E-cadherin expression and that reduces cell migration, this treatment seems to be a candidate for reducing metastasis, the principal lifethreatening complication of this type of cancer.

1-C Invasion pattern analysis of adenocarcinoma pancreatic PANC 1 cells after treatment with EHop 16 and 6 Shogaol

Natalia Acevedo, Luis Fuentes, Kevin Melendez Yazmín Reyes, Christopher Marrero, and Karen Woolcock

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer related death in the United States due to its invasive characteristics Cell invasion requires proteins that can degrade components of the extracellular matrix (allowing them to reach other parts in the body leading to metastasis Proteins RAC 1 and CDC 42 from the Rho family of GTPases control cell migration and other cellular processes. Other proteins like matrix metalloproteinases (degrade components of the extracellular matrix. The objectives of the investigation were to optimize the conditions for the invasion assay and to evaluate the effects of EHop 16 a Rac inhibitor, and 6 Shogaol on the invasive capacity of PANC 1 cells. Invasion assay was done using Boyden chambers placed on 24 well plates with EHop 16 10 m M) or 6 Shogaol 10 m M) with or without serum. Invasive cells were fixed, stained and counted and an average was determined. It was observed that exposure of PANC 1 cells to 6 Shogaol 10 μM) significantly reduced its invasion capacity by 24. This observation may be due to the reduction of MMP, an essential protein required for extracellular matrix degradation. Treatment with EHop 16 also reduced PANC 1 cell invasion capacity but only by 16. Therefore, interrupting the rearrangement of cytoskeletal through Rac inhibition has a limited effect on invasion. Pancreatic cancer cells may depend more on their secretion of MMP rather than on actin rearrangements. The effect of combining both compounds on cell invasion should be evaluated, since we have previously demonstrated that 6 Shogaol and EHop 16 has a synergistic effect for reducing PANC 1 cell proliferation.

1-D Evaluation of the pharmacological action of gemcitabine and natural compounds on cell cycle progression of PANC-1cells

Giancarlo Torres-López, Sheilin Martínez-Negrón, Neisha Ramírez-Serrano, Fabián Mercado-Nieves and Karen Woolcock

Pancreatic Ductal Adenocarcinoma has been a challenge over the years due to no symptoms until an advanced stage. In many cancer types, including PDAC the cell cycle is deregulated, a process controlled by the interaction of cyclins and CDKs. CDKs inhibition, has emerged as a potential therapy method for cancers. Several natural compounds from plants has been shown to inhibits CDK. Natural treatment 6-shogaol is responsible for the anti-inflammatory effect and anti-cancer activity. EHop-16 is also considered an inhibitor of the activity of cancer cells in the process of metastasis and proliferation. In this study, was evaluated the pharmacological effect of both treatments, natural and synthetic, on the progression of the cell cycle. Results showed that 6-shogaol had a net effect of increasing the percent of PANC-1 pancreatic cancer cells in S phase by 45%, while a net reduction was observed on the population of cells on G0/G1 phase and G2/M phase. In contrast it was observed that EHop-16 didn't alter the cell cycle stages in comparison with 6-Shogaol. The analysis of pharmacological effects of 6-Shogaol and EHop-16 on the cell cycle progression on PANC-1 cells, showed the potential effect of 6-Shogaol of halting the progression of the cell cycle. Since PDAC still a challenge, for that reason Cell Cycle Analysis on compound effect in PANC-1 cell suggest further experiments are needed for future result on the cyclin-dependent kinases (CDK)/cyclin complex.

1-E Migration pattern analysis of PANC-1 cells after treatment with EHop-16 and 6-Shogaol
Willton M. Antigua, Luis M. Fuentes, Gerardo L. Laureano, Claraisel Rodríguez, Myraida Toledo and Karen Woolcock

Pancreatic Ductal Adenocarcinoma (PDAC) is fourth most frequent cause of cancer related deaths and its overall 5-year survival is less than 8% due to its highly metastatic nature. Migration is critical step of metastasis that involves dynamic changes in the actin cytoskeleton and formation of lamellipodium and filopodium regulated by GTPase protein such as Rac1 and CDC42. Also, migration requires the participation of metalloproteinase such as MMP9. It has been demonstrated that 70% of pancreatic cancers have an overexpression of Rac1 GTPase and that pancreatic cancer has high expression of MMP-9. The aim of this study was to analyze EHop-16 and 6- Shogaol effect on the migration capacity of PANC-1 cell line. For this a Wound Healing Assay (WHA) was performed using a monolayer of PANC-1 cells grown in 6-well plate, with a system of coordinates. Result shown that EHop-16 (10 μM) had a net effect of reducing the migration 65.18% after 4 hrs of treatment, these was also observed with 6-shogaol (10 μM) which reduce cell migration 88.71% at 4 hrs. Both effects demonstrated with a 2way ANOVA to be statistically significant. The obtained results suggest that both 6-Shogaol and EHop-16 are possible options for treating PDAC by reducing metastasis, the principal life-threatening complication of this type of cancer.

ECOLOGÍA/ ECOLOGY

2-A Characterizing the coral reef fish assemblages among shallow water reefs from northern Puerto Rico
Gabriel Torres, Eduardo Hernández, Cesar Molina, Daniel A Toledo, Ernesto F Weil, Nikolaos V Schizas and Matthew Q Lucas

Reef fish play important ecological roles in the overall function and health of coral reefs Herbivorous fish maintain the balance between algal and coral growth while carnivorous fish keep herbivorous populations in check Coral reef health is not only determined by the abundances of fish that feed on them, but also by the different populations that do so. Overfishing, water pollution, and climate change factors have caused significant declines in reef fish assemblages in the Atlantic/Caribbean. In Puerto Rico, overfishing is one of the biggest threats to fish populations. Here, we aim to quantify reef fish assemblages among shallow water reefs by acquiring in situ data on reef fish abundances, diversity, density, and size class structure of reef fish from the northwestern and north central Puerto Rico We are currently collecting data on fish abundances, distributions, densities, species richness, and size class structure A total of ten shallow water 20 m depth) locations from northwestern and north central Puerto Rico will be assessed on SCUBA Reef fish will be video recorded along ten, belt transects 50 x 2 m) using a Nikon Coolpix W 300 (HD 4 K) camera mounted on a PVC constructed Tbar. In situ observations thus far, reveal a diverse range of fish species from numerous families including Pomacentridae Acanthuridae, Gobiidae Haemulidae Balistidae Lutjanidae as well as Elasmobranchs, among others Descriptive statistics (i e mean, variance, standard deviation, standard error) will be estimated for each location. Multivariate statistics will be used to assess management priorities in north central and northwestern Puerto Rico. This study will provide baseline data for local coral reef managers to implement and improve existing conservation efforts in these areas Long term monitoring of fish communities will determine if conservation efforts of these areas will stabilize reef fish assemblages and the overall health.

2-B Exploring the microbial associations and genetic diversity in *Dendrogyra cylindrus* (Scleractinia: Meandrinidae) from different environments
Genesis Pérez Cartagena, Norberto Rodriguez, Alok Arun, Daniel Toledo, Ernesto F. Weil, Nikolaos V. Schizas, and Matthew Q. Lucas

Scleractinian corals associate with a diverse microbiome including bacteria, archaea, viruses, fungi, and fundamental to their evolutionary success, photosynthetic dinoflagellates (family Symbiodiniaceae). Thermal stress increases susceptibility to pathogens and can destabilize the coral microbiome, sometimes leading to disease, bleaching, and mortality. The gap in our understanding of the mechanisms by which the coral microbiome supports coral health and increases resistance is substantial. We need to understand the relative contribution of the coral-host, the local environment, and thermal history in shaping microbial communities, as well as the role of Symbiodiniaceae in coral-holobiont adaptation to warming oceans. Listed as threatened, Dendrogyra cylindrus is a widespread, but low-abundance species due to low juvenile survivorship and recent mortalities caused by disease, bleaching, hurricanes, and local environmental degradation. This study aims to understand the effect of local environment, thermal history (SST), and coral-host (mitochondrial and nuclear lineages) on the bacterial and Symbiodiniaceae associations in D. cylindrus from two different environments, the north central (Arecibo) highenergy oceanic coast, as opposed to the calm, southwestern coast (La Parguera) of Puerto Rico. We are currently analyzing coral-host mitochondrial (NAD51d) and nuclear DNA (Calmodulin) intron sequences to estimate genetic diversity and construct Maximum-likelihood trees inferred in RaxML. Future work includes highthroughput sequencing of the bacterial (16S rRNA) and Symbiodiniaceae (ITS2) diversity. Multivariate statistics will be used to explain coral-host and microbial sequence data in colonies sampled from different environments. Collectively, microbial communities play important roles in ecological resilience. These data will serve as an important baseline on the microbial contributions, both bacterial and Symbiodiniaceae, for future studies in D. cylindrus. Further understanding the role of the coralholobiont, their environmentally-related differences, and their influence on coral resilience and adaptation are important for species conservation and coral reef management.

2-C Exploring the algal endosymbiont (family: Symbiodiniaceae) assemblages and genetic diversity in the threatened coral species *Dendrogyra cylindrus* (cnidaria: scleractinia) from different environments

Juan A. Santiago Rosario, Arismar Menieur, Jonathan González Vélez, Frances Coira Salgado, Kimberly Rivera, Alok Arun, and Matthew Q. Lucas

Scleractinian corals associate with a diverse microbiome including bacteria, archaea, viruses, fungi, and algal endosymbionts - deemed the “coral-holobiont”. However, fundamental to their evolutionary success over the past 250 million years is their symbiosis with photosynthetic algae of the family Symbiodiniaceae. The coral provides algal endosymbionts with a home and biological compounds (e.g., CO2, N, P) needed for photosynthesis, and in return, the algae produces oxygen and sugars for the coral animal as well as removing wastes. Coral-algal endosymbionts provide >95% of the daily carbon intake for the coral through its photosynthetic by products and therefore are critical to their growth (i.e., calcification), health, and survival. To this end, different coral-algal endosymbiont “species” in the family Symbiodiniaceae impart different physiological tolerances to environmental stressors such as turbid water that impedes light transmission for photosynthesis, nutrient pollution, and thermal anomalies related to ocean warming . Thus, we need to better understand the relative contribution of the coral-host, the local environment, and thermal history in shaping algal-endosymbiont assemblages in different coral species as well as the mechanisms in adapting to warming oceans. The gap in our understanding of these mechanisms by which algal endosymbionts (and the coral microbiome) supports coral health and increases resilience in different environments is substantial. This study aims to understand the effect of the local environment, thermal history (SST), and coral-host (nuclear DNA lineages) on the current algal-endosymbiont assemblages in Dendrogyra cylindrus from two contrasting environments, the north-central (Arecibo) high-energy oceanic coast, as opposed to the calm, southwestern coast (La Parguera) of Puerto Rico. Listed as threatened by the IUCN, D. cylindrus is a widespread, but low-abundance species due to low juvenile survivorship and recent mortalities caused by disease, bleaching, hurricanes, and local environmental degradation. To

assess the coral-algal assemblages (i.e., Symbiodiniaceae) in D. cylindrus, the ribosomal RNA (ITS2) region was PCR amplified and (~500 bp) will be analyzed with clustering analysis under a 97% sequence similarity threshold. To assess genetic diversity in D. cylindrus, PCR amplification of the coral-host nuclear DNA (Calmodulin) intron (~ 500 bp) will be analyzed with RaxML by constructing Maximum-likelihood trees. Multivariate statistics will be used to explain sequence data from D. cylindrus and its algal endosymbiont assemblages in the ten colonies sampled (n=5, north-central vs. n=5, southwestern PR). Future work includes Sanger sequencing of these PCR products and analyses of next—generation amplicon sequences of the bacterial (16S rRNA) community from the same coral samples used in this study. Overall, coral-algal endosymbionts (and others) play important roles in ecological resilience. These data serve as an important baseline on the symbiotic contributions, both the algal-endosymbionts and the bacterial community for future studies in D. cylindrus. Further understanding the role of coral-symbioses (i.e., coral holobiont), the influence of environmental differences on their symbiotic assemblages, and their role in coral resilience and adaptation are essential for species conservation and coral reef management.

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