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TITLE:

Investigating the 'Uncatchable Smile' in Leonardo da Vinci's La Bella Principessa: A Comparison with the Mona Lisa and Pollaiuolo's Portrait of a Girl

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SHORT ABSTRACT:

This paper discusses the methodology used to reveal the 'Uncatchable Smile' illusion in Leonardo da Vinci's La Bella Principessa portrait. A combination of three methods was used (inter-observation, structured interviews, and psychophysical experiments), which led to an investigation that shaped itself without prior beliefs, thus reducing potential researcher bias.

LONG ABSTRACT:

This paper discusses how the 'Uncatchable Smile' illusion in Leonardo da Vinci's La Bella Principessa portrait was discovered. Kemp and Cotte¹ described the expression of the Princess as ambiguous and "subtle to an inexpressible degree". A combination of three methods was used (inter-observation, structured interviews, and psychophysical experiments) to identify what may underlie this 'ambiguity'. The inter-observation and the structured interview methods were firstly applied to generate experimental hypotheses that were successively tested by a series of psychophysical experiments. The combination of these research methods minimizes the impact of the researcher's beliefs and biases in the development of the research design. It emerged that the ambiguity in La Bella Principessa is triggered by a change in the perceived level of contentment in her facial expression and that this perceptual change is attributable to a visual illusion relating to her mouth. Moreover, it was found that a similar effect can be observed in the Mona Lisa. As the smile in La Bella Principessa disappears as soon as the viewer tries to 'catch it', we named this visual illusion the 'Uncatchable Smile'. The elusive quality of the Mona Lisa's smile² is probably why the portrait is so famous, and so the existence of a similar ambiguity in a portrait painted by Leonardo prior to the Mona Lisa is even more interesting.

INTRODUCTION:

This paper discusses the methodology used by Soranzo and Newberry³ to investigate the 'Uncatchable Smile' illusion in Leonardo da Vinci's La Bella Principessa portrait. The overall goal of the methodology employed was to identify what makes the expression of the Princess ambiguous. The portrait shows the profile of a young woman identified as Bianca, the illegitimate daughter of Ludovico, who at around 13 years of age was to be married to a commander of the Duke's Milanese forces⁴. According to Kemp⁵, the portrait was commissioned by the Duke in honour of the celebration of her marriage in 1496. Kemp and Cotte⁶ noted that the expression of La Bella Principessa is ambiguous and "subtle to an inexpressible degree" (p.26), although the question remains as to what it is that triggers this ambiguity and whether this is similar to that perceived in Leonardo's Mona Lisa.

Soranzo and Newberry's³ study involved three stages which combined three different research methods. The rationale for using these different research methods was to conduct a research investigation that developed without prior beliefs, thus minimizing potential researcher bias. The first stage used the inter-observation method to investigate why the expression of the Princess is ambiguous; in the second, a structured interview was used to identify the psychological dimension underpinning the 'Uncatchable Smile' illusion; and in the third stage experiments were conducted to test hypotheses formulated on the basis of the findings of the first two stages. Independent groups of participants took part in these three stages and the findings of each stage influenced the following stages. This is likely to have reduced researcher bias compared to if the researchers had determined the research questions themselves.

The advantages of combining different research methods over singular methods are as follows. First, the inter-observation stage enabled discussion to take place about where the ambiguity may have originated from (rather than the researchers imposing their own predetermined view). Second, the interview stage enabled the identification of the psychological dimension to be investigated without imposing pre-defined categories. Third, the experimental stage enabled the perceptual change identified in the previous stages to be quantified in a controlled manner.

Inter-Observation

As noted above, La Bella Principessa's expression is "subtle to an inexpressible degree". The first step of the research was to try to identify what it is about the portrait that makes the expression ambiguous. To do this, the inter-observation method posited by Paolo Bozzi and his colleagues^{7, 8} was conducted with three participants and the senior author. This method enables maximum information to be extracted from direct experience⁹ and involves looking together, discussing and mutually exchanging views. The first step of the research adhered to the guidelines provided by Bozzi who argued that observers should conjointly partake in the observational process in a natural environment (not in a laboratory); observers should be aware of the researcher's hypothesis (no hidden hypothesis); the instructions to participants should be broad and discussed together rather than providing strict instructions; the discussion should go beyond the first impression (for a discussion of the un-permeability of this method in relation to the so called "conformity effect" found by Asch¹¹ see Bozzi⁷). According to Bozzi, the discussion should go beyond the first impression in order to explore all possible solutions. The conclusion is therefore reached through a process of "negotiation" between the participants and the researcher, and negotiation is the process through which a common account is determined¹⁰. Participants were presented with a copy of La Bella Principessa's portrait resting on an easel in diffuse lightning. General instructions were given; participants were requested to discuss the ambiguous expression of La Bella Principessa. In

the negotiation stage, it became clear early on that the ambiguity in the Princess's expression, or the allure of the portrait, is triggered by a perceptual change. The Princess's expression is ambiguous because it is unstable; sometimes she looks happy and cheerful whilst at other times she looks melancholic and hostile. However, the conditions under which this change occurs were still not known. Further inspection and negotiation led to the unanimous conclusion that the change relates to the way the portrait is viewed: the Princess's expression appears to change when she is viewed from different distances. When the Princess is viewed from far away her mouth appears to take an upward direction, making her appear friendly and positive, but as soon as the portrait is approached, the Princess's mouth appears to take a downwards slant, making her look sad and unapproachable. In other words, it seems that the ambiguity in the Princess's expression is due to a visual illusion of direction, which depends on the viewing distance. Since spatial frequency changes with distance it was concluded that this change relates to spatial frequency.

The existence of a spatial frequency-dependent illusion of direction was also explored by using a blurred digital version of the portrait. A digital version of La Bella Principessa was presented on a computer screen and the level of blur was manipulated. The blurring process was essentially a convolution kernel of the pixel spreading type in which pixels take contributions from their surrounding pixels, while de-emphasising contributions from the centre¹². In addition to appearing more cheerful and happy when viewed from a distance, La Bella Principessa also appeared more cheerful and happy when viewed as a blurred image. This supports the idea of a spatial frequency-dependent illusion. The concept of spatial frequency-dependent facial expression is supported by a wide understanding of how the human visual system is particularly sensitive to seeing and interpreting the subtleties of facial expression^{13, 14}.

Structured Interviews

The results of the inter-observation stage led us to hypothesize that the Princess's expression is ambiguous because it is unstable and that this instability may be attributable to a spatial frequency-dependent perceptual change relating to the direction of the mouth. The assumption was that if there is a 'perceptual change' which relates to the way the portrait is viewed, then there must be a controllable way to elicit one impression in the viewer over another (for example, the melancholic one rather than the cheerful one). The challenge was to find out how to control the viewer's impression of La Bella Principessa in order to generate one impression over the other. Before addressing this problem in a quantitative manner, structured interviews were conducted to determine the psychological dimension that best captures this perceptual change.

Four participants who had not taken part in the inter-observational stage of the study took part in a structured interview. Participants were shown an identical copy of La Bella Principessa's portrait resting on an easel in diffuse lighting. Participants were asked to describe the Princess's expression and to note any differences in the expression when viewed from close or further away. Interestingly, participants' descriptions were not framed in terms of outward expressions such as 'smiling' but more with the internal emotional state of the Princess. A Grounded Theory analysis 15,16,17,18 of the descriptions was conducted and it was found that the most common words used to describe the Princess's expression were "happiness" and "contentment". These findings suggested that the change in the Princess's expression might be better captured with the definition of a change in perceived "contentment". Together, the inter-observational and structured interview stages informed the formulation of five hypotheses as follows: First, the viewer's distance from La Bella

Principessa's portrait will influence their perception of her facial expression. Specifically, the ratings of contentment will be higher when the portraits are viewed from further away. Second, the portrait's level of blur will influence the perceived contentment of La Bella Principessa. Specifically, presenting digital versions of the portrait with an increased level of blur will lead to an increase in the viewer's perception of contentment. Third, the uncatchable smile illusion is mainly attributable to the mouth area of La Bella Principessa's portrait. Fourth, the perceived slant of La Bella Principessa's mouth will change when the distance of the portrait is manipulated. Specifically, the corner of the mouth will appear to take an upward turn when distance is increased. Fifth, the perceived slant of La Bella Principessa's mouth will change when a digital version of the portrait is presented with different levels of blur. Specifically, the corner of the mouth will appear to take an upward turn when the level of blur is increased.

Psychophysical Experiments

In the quantitative stage of the study, five psychophysical experiments were conducted to test the aforementioned hypotheses. In addition, since it was hypothesized that a similar effect would be observed for the Mona Lisa's expression, this portrait was also included in the investigation. Furthermore, to test whether the effect under investigation is specific to La Bella Principessa and the Mona Lisa or whether it may be general to all portraits of Leonardo's era, a control portrait was also used. This was the Portrait of a Girl painted around 1470 by Piero del Pollaiuolo. This was chosen as a suitable control for La Bella Principessa as the portrait is from the same period and there are similarities of size, presentation and appearance in the subject matter.

PROTOCOL:

The protocol was approved by the Faculty Research Ethics Committee at Sheffield Hallam University for studies on human subjects.

1. Inter-observation stage

- 1.1) Identify how and why La Bella Principessa's expression appears ambiguous.
- 1.1.1) Display a good quality, frameless and exact sized foam backed digital copy of La Bella Principessa (33.2 cm in height x 23.8 cm in width) on an easel in diffuse lighting.
- 1.1.2) Have four participants discuss the ambiguous expression in La Bella Principessa's portrait. Record the results of the discussion on a piece of paper.

Note: One participant must be the researcher and the others must be recruited from the academic community.

Note: Ensure that the participants can freely approach the portrait from any distance or angle.

2. Structured interview stage

2.1) Interview four different participants one at a time and have them answer the questions contained within the structured interview schedule: "Please can you describe the Princess's expression? Do you think there are any differences in the Princess's expression when you view her from close or further away? If so, please explain your answer".

- 2.1.1) Display La Bella Principessa's portrait (33.2cm H x 23.8cm W) in the centre of an A1 paper pad resting on an easel in a well illuminated room.
- 2.1.1.1) Instruct the participants to describe the princess's expression (e.g. happy or melancholic) and note any differences in the expression when viewed from close-up (to within a few centimetres of the portrait) or further away (more than three meters away) from the portrait. Have the participants write down their observations on paper.
- 2.2) Conduct a Grounded Theory analysis ^{15,16,17,18} of participants' descriptions to identify the most common words used to describe La Bella Principessa's expression (e.g. smiling, happiness, contentment) and changes to her expression with respect to distance (e.g. less content when closer, etc.).

3. Psychophysical experiments stage

- 3.1) Conduct five experiments to test the five hypotheses formulated on the basis of findings from steps 1 and 2, as outlined below.
- 3.1.1) On Viewing Distance (experiment 1)
- 3.1.1.1) Conduct a between-subjects experiment with two independent variables (IVs): Portrait (with three levels: La Bella Principessa, Mona Lisa, and Portrait of a Girl); and Viewing Distance (with two levels: Close vs. Far).

Note: The dependent variable must be perceived contentment (i.e., how much on the scale of 1 to 7 the women in the portraits appear content).

- 3.1.1.1.1) Recruit 60 participants, 10 for each of the six conditions resulting from the combination of the factor levels indicated in 3.1.1.1. Ensure that the participants have normal or corrected-to-normal acuity (determined by self-reporting) and are naive with regard to the experimental design. Ensure that the participants are not familiar with either La Bella Principessa or the Portrait of a Girl and have not seen the Mona Lisa for at least one year.
- 3.1.1.1.2) Display good quality and exact sized foam backed digital copies of La Bella Principessa (33.2cm H x 23.8cm W), Mona Lisa (77 x 53cm), and Portrait of a Girl (45.5 x 32.7cm) in the centre of an A1 paper pad resting on an easel 1.80 metres from the floor.
- 3.1.1.1.3) Mount the portraits indicated in 3.1.1.1.2 in diffused lighting at the end of a long corridor with two doors 0.5 and 8 meters from the portraits, respectively, and cover them with a sheet. Place two markers on the floor, one 0.5 meters from the portrait for the Close condition, and the other 8 meters from the portrait for the Far condition.
- 3.1.1.1.4) Randomly assign participants to the Close or Far condition and to one Portrait condition. Have the participants in the Close condition enter the corridor from the closest door, and the participants assigned to the Far condition enter the corridor from the furthest door.

- 3.1.1.1.5) Have the participants stop at a location indicated by the marker on the floor and then unveil the portrait. Ensure that the participants are able to see the portrait from the assigned marker only and not experience any change in the viewing distance.
- 3.1.1.1.6) Have the participants verbally rate the perceived contentment in the expressions of the woman viewed in the assigned portrait on a scale of 1 (not content at all) to 7 (extremely content). Record participants' responses on a piece of paper (e.g. Participant 1; Close Condition; Mona Lisa; Contentment = 5).
- 3.1.2) On Blur Level (Experiment 2)
- 3.1.2.1) Conduct a within subjects experiment (i.e. each subject is presented with all the experimental conditions) with two IVs: Portrait (with three levels: La Bella Principessa, the Mona Lisa, and Portrait of a Girl); and Level of Blur (with eight levels of increasing blur).

Note: The dependent variable must be perceived contentment.

- 3.1.2.1.1) Recruit 10 participants who have not taken part in any previous stages of the study.
- 3.1.2.1.2) Create a set of eight digital images for each of the three portraits with increasing levels of Gaussian Blur ¹². Ensure that the Blur level increases in steps of 1 pixel over the range 0-7.

Note: In this blurring process pixels take contributions from their surrounding pixels and contributions from the centre are de-emphasised.

- 3.1.2.1.3) Set up a 22" (20" viewable) CRT monitor set at a resolution of 1280 x 1024 connected to a computer.
- 3.1.2.1.4) Write software¹⁹ (see Supplemental file) that displays the portraits in a random order on the monitor; presents a random noise mask (i.e., a figure made by black and white 1 pixel large dots randomly distributed across the screen) for one second before the presentation of each picture; allows participants to enter their ratings of contentment from 1 to 7 using a keyboard; and records the ratings when the spacebar is pressed.

Note: See supplemental "Code for exp 2.bak" file for an example on how this could be programmed in True Basic¹⁹, although other programs can be used.

- 3.1.2.1.5) Seat participants one at a time in a well-illuminated room 50 cm from the monitor.
- 3.1.2.1.6) Have the participants rate the perceived contentment in the expression of La Bella Principessa, Mona Lisa and Portrait of a Girl from 1 to 7 and enter their ratings using a keyboard. Record the responses when the spacebar is pressed as per step 3.1.2.1.4 above.
- 3.1.2.1.7) To eliminate any influences from the previous images affecting participants' ratings of the new ones, present a random noise mask full screen that disappears automatically after one second for one second prior to the next image being shown. Allow the image to remain on the screen for as long as the participant desires.
- 3.1.3) On Establishing the Source of the Illusory Effect (Experiment 3)

3.1.3.1) Conduct a repeated-measures experiment with two IVs: Level of Blur (with eight levels); and Mask Position (with four levels: No mask, Mouth mask, Eye mask, or both Mouth and Eye masks).

Note: The dependent variable must be perceived contentment.

- 3.1.3.1.1) Recruit 10 participants who have not taken part in any previous stages of the study.
- 3.1.3.1.2) Use the same digital images of La Bella Principessa with different levels of Gaussian blur used in experiment 2. Using graphical software (e.g., Photoshop), create a solid black rectangle of 0.3×0.6 cm and place it over the mouth, or the eye or over both the mouth and the eye.
- 3.1.3.1.3) Create three more sets of masked images to result in a total of four sets (each comprising eight images): For the first set create eight images for the No Mask condition; for the second set create eight images for the Mouth Mask condition; for the third set create eight images for the Eye Mask condition (of La Bella Principessa's visible eye); and for the fourth create eight images for the Eye and Mouth condition.

Note: The mask itself must not be blurred. See supplemental "Code for exp 3.bak" file for an example on how this could be programmed in True Basic, although other programs can be used.

- 3.1.3.1.4) Use a CRT monitor and the same conditions as for experiment 2. Have the participants rate the perceived contentment of La Bella Principessa on a scale of 1 (not content at all) to 7 (extremely content) by pressing the correspondent key on the numeric keypad.
- 3.1.4) Distance on Perceived Mouth Slant (Experiment 4)
- 3.1.4.1) Conduct a between-subjects experiment (i.e. different subjects for the different conditions) with one IV (Viewing Distance with two levels: Close vs. Far).

Note: The dependent variable must be the perceived slant of the mouth.

- 3.1.4.1.1) Recruit 20 participants (10 in the Close Condition and 10 in the Far condition) who have not taken part in any previous stages of the study.
- 3.1.4.1.2) Project an exact sized (33.2cm H X 23.9cm W) digital version of La Bella Principessa using a projector onto a screen connected to a computer at 1.80 metres from the floor in a large room with diffused lighting.
- 3.1.4.1.3) Place a wireless keyboard on top of a table at 0.5 meters from the screen for the Close Condition. Place the wireless keyboard on top of a table 8 meters from the screen for the Far Condition.

Note: Ensure that the sizes of the retinal images of the portrait in degrees of visual angle are the same as for experiment 1. Ensure that the participants view La Bella Principessa from the assigned location only and do not experience any change in the viewing distance.

- 3.1.4.1.4) Using the programming software described above (3.1.3.1.3), create a 0.9 cm long black hairline measurement 'handle' on the screen (the same length as La Bella Principessa's mouth). Place the hairline at the same level and 4 cm to the left of the mouth.
- 3.1.4.1.5) Make the right side of the handle adjustable by pressing a button of a wireless mouse and record the perceived mouth slant angle when the spacebar key is pressed (see supplemental "Code for exp 4.bak" file).
- 3.1.4.1.6) Have the participants to adjust the 'handle' using the mouse. Instruct participants to press the mouse button to adjust the slant of the handle until it aligns with the perceived slant of the mouth of the woman in the portrait. Leave the images on the screen for as long as the participant desires.
- 3.1.5) Blur on Perceived Mouth Slant (Experiment 5)
- 3.1.5.1) Conduct a repeated-measures experiment with one IV (Blur with eight levels).

Note: The dependent variable must be the Perceived slant of the mouth.

- 3.1.5.1.1) Recruit 10 participants who have not taken part in any previous stages of the study.
- 3.1.5.1.2) Using the same apparatus used for experiment 2 and the same on-screen facility used in experiment 4, present the eight versions of La Bella Principessa with different levels of blur in a random order on the screen (see supplemental "Code for exp 5.bak" file).
- 3.1.5.1.3) As for experiment 4, instruct participants to adjust the slant of the 'handle' until it aligns with the perceived slant of the mouth of the woman in the portrait. As in experiments 2 and 3 present a random noise mask on the screen one second prior to the next image being shown. Leave the images on the screen for as long as the participant desires.

Note: The software records the perceived mouth slant angle when the spacebar key is pressed.

REPRESENTATIVE RESULTS:

From the negotiation process of the Inter-observation stage it was ascertained that the ambiguity in the Princess's expression might be due to a visual illusion of direction which depends on the viewing distance. Since spatial frequency changes with distance it was concluded that this change relates to spatial frequency. A Grounded Theory analysis of the descriptions obtained in the Structured Interview stage was performed and it was found that observers tended to describe her change of expression in terms of contentment. Therefore, the term "contentment" was adopted as the dependent variable for the subsequent experimental stage. Five psychophysical experiments were conducted to test the hypotheses generated in the previous qualitative stages. Experiment 1 supported Hypothesis 1: When La Bella Principessa and the Mona Lisa were observed from a distance their Perceived Contentment was significantly higher than when the portraits were viewed from close-up, whereas there was no significant effect for Pollaiuolo's Portrait of a Girl (see Figure 1). Specifically, in the Far condition the average Perceived Contentment for La Bella Principessa and the Mona Lisa was about 2 and 1.5 units higher (in the range 1-7) rather than in the Close condition, respectively;, whilst there was no significant difference for Pollaiuolo's Portrait of a Girl.

This finding supported Hypothesis 1. Second, the level of blur affected the Perceived Contentment of La Bella Principessa and the Mona Lisa but not the Perceived Contentment of Pollaiuolo's Portrait of a Girl. As can be seen in Figure 2, increasing the level of blur increased Perceived Contentment in a linear fashion, whilst this experimental manipulation did not affect the Perceived Contentment of Pollaiuolo's Portrait of a Girl. This finding supported Hypothesis 2. Third, the Perceived Contentment of La Bella Principessa increased with the Level of Blur in both the No Mask and Eye Mask conditions whereas there was no significant linear trend between Level of Blur and Perceived Contentment for either the Mouth and both Mouth and Eye mask conditions (see Figure 3). This supported Hypothesis 3 that the uncatchable smile illusion is mainly attributable to the mouth area of the portrait. Fourth, the Perceived Mouth Slant of La Bella Principessa changed with viewing distance; her mouth was perceived to take an upward slant when viewed from a distance, whereas it was perceived to take a downward direction when observed from close-up (see Figure 4). Specifically, in the Close condition the mouth was perceived to take a downwards direction (an average of about 7.5 degrees), whilst in the Far condition, the mouth was perceived to take an upwards direction (an average of about 1 degree). This finding supported Hypothesis 4. Finally, the Perceived Mouth Slant of La Bella Principessa changed with Level of Blur; when viewed with an increasing level of blur her mouth was perceived to take a more upward slant (see Figure 5). Specifically, the mouth slant was perceived to take a downwards slant (an average of about 8 degrees) when no blur was applied; and it took a linear upwards direction when the level of blur was gradually increased. This supported Hypothesis 5.

Figures 1 to 5 show examples of results from Soranzo & Newberry³ to illustrate how the findings from the experiments could appear.

FIGURE		HERE
FIGURE	2	HERE
FIGURE	3	
FIGURE	4	HERE
FIGURE		

Figure Legends:

Figure 1. The effect of distance on the perceived contentment of La Bella Principessa, Mona Lisa and Portrait of a Girl. This figure shows how distance could influence the perceived contentment of La Bella Principessa, Mona Lisa and Portrait of a Girl. Bars indicate standard errors. This figure has been modified from Soranzo and Newberry³.

Figure 2. The effect of blur on the perceived contentment of La Bella Principessa, Mona Lisa and Portrait of a Girl. This figure shows how blur could influence the perceived contentment of La Bella Principessa, Mona Lisa and Portrait of a Girl. Dotted lines favour the view of the trend. Bars indicate standard errors. This figure has been modified from Soranzo and Newberry³.

Figure 3. The effect of blur and masking on the perceived contentment of La Bella **Principessa.** This figure shows how blur could affect the perceived contentment of La Bella Principessa when different parts are masked. Bars indicate standard errors. This figure has been modified from Soranzo and Newberry³.

Figure 4. The effect of distance on the perceived mouth slant in La Bella Principessa's portrait. This figure shows how distance could influence the perceived mouth slant in La Bella Principessa's portrait. Bars indicate standard errors. This figure has been modified from Soranzo and Newberry³.

Figure 5. The effect of blur on the perceived mouth slant in La Bella Principessa's portrait. This figure shows how blur could influence the perceived mouth slant in La Bella Principessa's portrait. Dotted lines favour the view of the trend. Bars indicate standard errors. This figure has been modified from Soranzo and Newberry³.

Supplemental file 1. Code for experiment 2.

Supplemental file 2. Code for experiment 3.

Supplemental file 3. Code for experiment 4.

Supplemental file 4. Code for experiment 5.

DISCUSSION:

The findings of the study conducted by Soranzo & Newberry³ indicate that the ambiguity of the expression in Leonardo da Vinci's *La Bella Principessa* portrait is attributable to a visual illusion. A new mixed-methodology which combined three methods: Inter-observation, structured interviews, and psychophysical experiments was adopted. Firstly, the inter-observation method was used to shed some light on the 'ambiguity' of the Princess's facial expression. This method which involves looking together, discussing and mutually exchanging views (Bozzi⁷), led the participants to realise that the expression, or the allure of the portrait is triggered by a perceptual change. This change seems to be related to the way the portrait is viewed: the Princess's expression appears to differ when she is viewed from different distances. Furthermore, it seems that this change of appearance can be attributed to a visual illusion of direction: the mouth appears to take an upward direction when viewed from distance (eliciting a cheerful expression), but it seems to take a downward direction when viewed from close-up (eliciting a hostile expression). Moreover, it has been noted that a similar change occurs by manipulating the level of blur in a digital version of the image.

Before conducting psychophysical experiments to test the hypotheses formulated from the findings of the inter-observation method, structured interviews were conducted to determine the psychological dimension responsible for the change of expression. Interestingly, participants' descriptions were not framed in terms of outward expressions such as 'smiling'

but more with the internal emotional state of the Princess, such as her level of "contentment". An advantage of the inter-observation and structured interview was that that these stages led to a clearer data-driven quantitative stage, which reduced speculation as to what the (potential) effect is or what construct(s) it relates to. Finally, psychophysical experiments were conducted which corroborated the hypotheses that both distance and level of blur influence La Bella Principessa's facial expression and that this effect can be attributed to a visual illusion relating to the direction of her mouth.

The presence of a visual illusion of direction that is dependent upon spatial frequency is very interesting and novel. Although numerous visual illusions of direction have been reported^{20, 21, 22}, to the best of our knowledge none of them are spatial frequency-dependent, at least in the "static" domain. A spatial frequency-dependent change of direction has been reported^{23, 24}, although these studies concern kinetic rather than static displays. The outcome of the inter-observational stage in Soranzo and Newberry's study³ indicates that the ambiguity in the Princess's expression might arise from an original spatial frequency- dependent *static* illusion of direction. Moreover, it was found that a similar effect can be observed in the Mona Lisa. The existence of a similar elusive quality in a portrait painted by Leonardo prior to the Mona Lisa is fascinating, especially considering that this quality is probably what makes the Mona Lisa so popular.

As La Bella Principessa's smile disappears as soon as the viewer tries to 'catch it' we have named this visual illusion the 'Uncatchable Smile'. By combining different stages of investigation, the study has significance with respect to existing methods because it enabled the identification of what generates the ambiguity in La Bella Principessa's expression (interobservation stage); the identification of the psychological dimension to be investigated (interview stage); and the perceptual change identified in the previous stages to be quantified in a controlled manner (experimental stage). The first two qualitative stages enabled testable hypotheses to be formulated without influence from the researchers' prior beliefs, and the third quantitative stage enabled the hypotheses to be tested in a controlled setting. Other studies have investigated facial expressions and emotion using forced-choice response formats. For example, in Ambadar, Schooler and Cohn's study ²⁵, participants were requested to select the emotion that best described different facial expressions from a range of predefined options. This wouldn't have been a suitable method in Soranzo and Newberry's study because offering a predefined set of options would have limited participant responses.

Some steps within the protocol must be emphasized. Different participants must be recruited for the three different stages of the method. Whilst in the inter-observation stage participants can be familiar with the stimuli, it is important that in the experimental stage participants are naive with regards the hypotheses. In addition, the stimuli (digital copies of portraits) must be of high quality resolution, and the experimental conditions must be carefully controlled, for example, the lighting conditions.

Some limitations of the protocol must be borne in mind. One limitation is the selection of participants for the Inter-observation stage. Individual differences may account for how the discussion evolves. For example, different levels of prior knowledge of art or perception could bias the discussion. As the aim of this stage is to conjointly develop the research question(s) through the process of "negotiation" (as defined by Bozzi⁷ and outlined in the introduction) the recruitment of academics as participants was considered to be the most suitable option since academics are more likely to question the issue in a critical and considered way. A limitation of the protocol for the structured interview stage is that the

questions could lead the participants to believe that there are differences in the Princess' expression when viewed from different distances. We considered this when formulating the questions (for example, we did not ask "Do you think the Princess looks happier when viewed from far away?"). A limitation of the experiments in the psychophysical stage that used the Likert scale is the range of response options. A scale of 1 to 7 was considered to be the most appropriate (1= not content at all to 7 = extremely content) because this seemed to better capture the range of possible emotion in the princess's expression. A more restricted Likert scale (e.g. 1= not content at all to 5=extremely content) may not have captured this variation, whereas a broader scale (e.g. 1 = not content at all to 9 = extremely content) may have led to an arbitrary increase of variability in responses.

In general, some modifications could enhance future replications of the study which would help address some limitations of the technique used. For example, whilst Pollaiuolo's Portrait of a Girl was considered a suitable control portrait for La Bella Principessa since it is from the same period and there are similarities of size, presentation (both were painted in profile) and appearance in the subject matter, it was not a suitable control for the Mona Lisa since the latter was painted face-on rather than in profile. It would therefore be valuable to include additional control portraits in future studies.

This multi-stage methodology can be adapted in future applications to study different illusory phenomena where the source of the effect is ambiguous. For example, it could be applied to investigations of other Leonardo da Vinci paintings and other artworks from that era. It may also be helpful in the investigation of visual illusions where there is uncertainty about what elicits a particular effect and where the phenomena are not clearly defined such as reverse figures, illusory movements, and after images.

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DISCLOSURE:

The authors have no competing financial interests.

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